PRESSURE-ASSISTED OZONATION AND AERATION OF ACTIVATED SLUDGE FOR CELL RUPTURING AND ANAEROBIC DIGESTION ENHANCEMENT

by

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ABSTRACT

The generation of a large volume of activated sludge (AS) from wastewater treatment has increasingly become a great burden on the environment. Anaerobic digestion is routinely practiced for excess waste sludge; however, the process retention time is long because of kinetic limitation in the hydrolysis step. This study tested the feasibility of applying cycles of pressure-assisted ozonation (PAO) to enhance the disintegration and solubilization of AS for digestion using reduced ozone dose and contact time. The kinetics of solid reduction and biogas production of PAO-treated sludge are evaluated in this study. The ultimate goal is to introduce an effective treatment method that produces increased soluble organic contents from AS for enhanced excess sludge reduction and biogas production during conventional anaerobic digestion. Results show that for the returned AS with total chemical oxygen demand (tCOD) of 8200 mg L^{-1}, a dose of 0.01 g O_3 g^{-1} total suspended solids (TSS) delivered via 20 pressure cycles within 16 min resulted in a 37-fold increase of the sCOD/tCOD ratio (due to increased soluble COD, i.e., sCOD) and a 25% reduction of TSS, in comparison to a dose of 0.08 g O_3 g^{-1} TSS via bubbling contact over 15 min that resulted in a 15-fold increase of the sCOD/tCOD ratio and a 12% reduction of TSS. Sludge solubilization was evidenced by increased dissolved contents of total phosphorous (from 10 to 64 mg L^{-1}), total nitrogen (from 14 to 120 mg L^{-1}), and protein (from < 15 to 39 mg L^{-1}) in the sludge suspension after treatment, indicating significant solubilization of AS. The subsequent anaerobic

digestion tests showed solids reduction to be significantly dependant on food-to-inoculum (F/I) ratios in the range 0.5 to 2 rather than treatments when unacclimated inoculum was used. However, improvements of solids reduction and biogas production by conventional ozonation and PAO resulted when using acclimated inocula at F/I = 0.4 to 0.8. Using PAO at a smaller dose (10 mg O_3 g^{-1} TSS) at F/I of 0.8, VSS reduction and biogas production were 1.4- and 2.6-fold higher, respectively, than those of conventional ozonation (52 mg O_3 g^{-1} TSS). This study has demonstrated the advantage of using PAO in disrupting excess activated sludge and in terms of improved solids reduction and biogas production during subsequent anaerobic digestion.

To my LORD, my family, and my friends.

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CHAPTER 1

INTRODUCTION

While the activated sludge (AS) process is universally applied for wastewater treatment with good outcomes, the process generates excess sludge. The sheer volume of excess sludge in cities around the world has become an increasing burden for the environment as well as precious land space, especially in densely populated urban centers (Bougrier et al. 2007; Chu et al. 2009b; Park et al. 2003; Weemaes et al. 2000b). Traditional applications of wasted AS, such as agricultural application (e.g., compost), land disposal, and incineration, entail significant environmental issues or cost (Bougrier et al. 2007). Anaerobic digestion of AS is commonly practiced to reduce the sludge volume and recover biogas, a form of renewable energy. However, a challenge remains with the slow kinetics of the hydrolysis reaction, the first step in fermentative biogas production (Pavlostathis and Gossett 1986). Benefits of sludge treatment prior to anaerobic digestion include a shorter digestion period, greater solids reduction, and increased energy production. Various treatment methods have been studied that are to be implemented between the primary clarifier and the digestion reactor of wastewater treatment plants (WWTPs) (Carrere et al. 2010).

Among different physical and chemical pretreatments, ozonation of sludge has shown promise in improving sludge treatability, available chemical oxygen demand (COD), and biogas production (Bougrier et al. 2007; Dogruel et al. 2007;

Weemaes et al. 2000a; Yasui et al. 2005; Yeom et al. 2002). While the mechanism of cell solubilization by ozone is not fundamentally understood, ozonation dosage and contact time are well recognized as primary factors for enhanced solubilization. Although ozonation pretreatment of AS has been tested in scales ranging from bench top to full wastewater treatment plant, only a few studies focused on low dosage for sludge ozonation (Chu et al. 2009a), such as with 23 μg O_3 g^{-1} volatile suspended solids (VSS) (Caravelli et al. 2006) and 13.6 mg O_3 g^{-1} COD of sludge (Dziurla et al. 2005). Researchers generally found 50 mg O_3 g^{-1} dry solids an optimal dose (Park et al. 2003; Zhang et al. 2009). Chu et al. (2009b) recently reviewed ozonation pretreatment for minimizing sludge production and concluded that ozone doses of 30 to 50 mg O_3 g^{-1} total suspended solids (TSS) would be technically and economically viable.

Cell solubilization, manifested as flocs disintegration, solids reduction, and soluble organics increases, was generally found when 50 or more mg O_3 g^{-1} dry solids was applied on activated sludge (Park et al. 2003; Zhang et al. 2009), and the extent of solubilization was typically associated with increased anaerobic biogas production (Braguglia et al. 2006; Weemaes et al. 2000b). However, treatment for mixtures of kitchen wastes and activated sludge exhibited no correlation between solubilization (e.g., soluble COD, i.e., sCOD, solubilization of 4-15%) and biogas production, which was attributed to refractory compounds released during treatment (Ma et al. 2011). Moreover, life cycle assessment (LCA) of anaerobic digestion has shown that ozonation of mixtures of kitchen waste and sewage sludge may not be beneficial but can consume more energy and exert greater environmental burdens than its benefits (Carballa et al. 2011).

Feed-to-inoculum ratio (F/I, typically VS-based) is an important factor when evaluating the effectiveness of biogas production and biodegradability (Braguglia et al. 2006; Jensen et al. 2011; Tomei et al. 2008). In some cases, due to formation of biodegradation intermediates and pH changes, the ratio of substrate/inoculum became a critical factor (Neves et al. 2004). For both untreated and sonicated sewage sludge, solids reduction and sludge hydrolysis were increased when F/I ratio was increased from 0.1 to 4, with most favorable F/I between 0.1 and 2 (Braguglia et al. 2006; Tomei et al. 2008). Although ozonation had been applied at pilot scale in WWTPs, the effect of F/I based on batch digestion study was not available.

A pressure-assisted ozonation (PAO) system was found very effective for removing organic contaminants from sediment and water (Cha et al. 2010; Hong et al. 2008b). The microbubbles were created by venting of the slurry that had been priorly oversaturated with the ozone/air mixture under pressure; thus created, the expanding gas bubbles ran the gamut of sizes from nano and micro to cm. Sediment aggregates were disrupted by pressure cycles into constituent fine-grained particles; thus, the contaminants in the sediment were more effectively removed (Hong and Nakra 2009). Chu et al. (2008) also employed mechanically generated ozone microbubbles of relatively static sizes to improve the effectiveness of AS treatment, and focused on high utilization of ozone, inactivation of microbes, and release of COD and other nutrients. Relative to microbubbles generated by mechanical means, the occurrence of expanding microbubbles via depressurization is ubiquitous and uniform throughout the reactor volume and not dependent on the location of an injector. For implementation, the new sludge treatment process is anticipated to be placed between the secondary clarifier and the digestion reactor of the wastewater treatment plant.

As the groundwork of ozonation in pressure cycles built previously, this study evaluates the technique's utility to enhance AS solubilization and solids reduction in comparison to conventional bubbling ozonation in terms of ozone dosage and contact time. The biodegradability of PAO-treated sludge in the batch anaerobic digesters was evaluated as well. The kinetics of solid reduction and biogas production of PAO-treated sludge are evaluated. The ultimate goal is to introduce an effective treatment method that produces increased soluble organic contents from AS for enhanced excess sludge reduction and biogas production during conventional anaerobic digestion.

CHAPTER 2

RESEARCH HYPOTHESES AND OBJECTIVES

2.1 Problem statement

The activated sludge (AS) process has been effectively applied for nutrients removal from wastewater; however, the sheer volume of excess waste sludge around the world has become an increasing burden for the environment. Although ozonation treatment of AS has been tested in scales ranging from bench top to full wastewater treatment plant, only a few studies focused on low dosage for sludge ozonation. There remains a need to reduce the burden of excess waste sludge or increase the biogas production. This can be accomplished by improving the solids reduction kinetics and biogas yield by improving the feed to the anaerobic digestion process. Another need is a better understanding of food-to-inoculum ratio that significantly influences digestion efficiency, particularly the effect when the sludge substrate has been priorly treated by ozonation.

2.2 Research objectives

This research will develop a method to treat activated sludge prior to anaerobic digestion and test the effectiveness of the method. An overall objective is to improve digestion kinetics, biogas production, and solids reduction. The first project objective is to promote cell solubilization with the new method and evaluate its effect under different conditions. The second objective is to determine the effects

of treatment in terms of solids reduction and biogas production along with other important factors such as the food-to-inoculum ratio being employed.

2.3 Research hypotheses and approach

2.3.1 Part I – Solubilization of activated sludge

Hypothesis 1: Ozonation ruptures activated sludge and solubilizes cell substances into the aqueous phase.

Hypothesis 2: Ozonation via rapid pressure cycles enhances rupture and solubilization by promoting more intense contact of ozone with sludge, resulting in increased soluble substance in the aqueous phase with lower dose and/or contact time.

In order to examine this hypothesis, major tasks were performed:

- Determine and evaluate the solubilization of COD and suspended solids before and after treatment
- Determine the changes of cellular materials (e.g., DNA, protein, soluble total nitrogen, and total phosphorus) in the aqueous phase before and after treatment
- Compare the solubilization efficiency of PAO treatment with that of conventional ozonation and others in the literature
- Explain the cell rupture mechanism by pressure-assisted ozonation

2.2.2 Part II – Enhanced digestion of treated activated sludge

Hypothesis 3: Feed of solubilized activated sludge (already ruptured sludge) enhances digestion kinetics, leading to improved solids reduction.

Hypothesis 4: Feed of solubilized activated sludge enhances biogas production by shortening the initial slow-step of cell hydrolysis (cell rupturing).

To examine the hypotheses, the following major tasks were carried out:

- Conduct batch digestion experiments with untreated and treated sludge and compare result of solids reduction and biogas production
- Compare the digestion results of untreated sludge with sludge treated with PAO and conventional ozonation
- Evaluate solids reduction and biogas production under different food-to-inoculum ratios

CHAPTER 3

LITERATURE REVIEW

3.1 Aerobic activated sludge and anaerobic digestion processes

Activated sludge (AS) processes in wastewater treatment plants for biological nutrient removal are universally practiced nowadays. For wastewater treatment, the AS process has been an effective biological treatment process for removal of organic matters and nutrients. Three key functions are present in the AS process: (1) biological nutrient removal, (2) liquid-solids separation, and (3) contents recirculation (Fig. 3.1). To maintain AS process performance, the selection of proper solids retention time (SRT) is critical. Adjusting the recycle ratio and wasting of some unwanted activated sludge are means of maintaining desirable SRT (Metcalf and Eddy Inc. 2004). Traditional applications of wasted activated sludge, such as agricultural application (e.g., compost), land disposal, and incineration, resulted in significant environmental or cost issues (Bougrier et al. 2006; Zhang et al. 2009). The increasing volume of excess sludge in cities around the world has become a burden for the environment as well as precious land space, especially in densely populated urban centers. In America and Europe, the amount of waste sludge is increased because of legal restrictions for effluent from wastewater treatment plants (Bougrier et al. 2006; Mines et al. 2008; Weemaes et al. 2000b). In Korea and China,

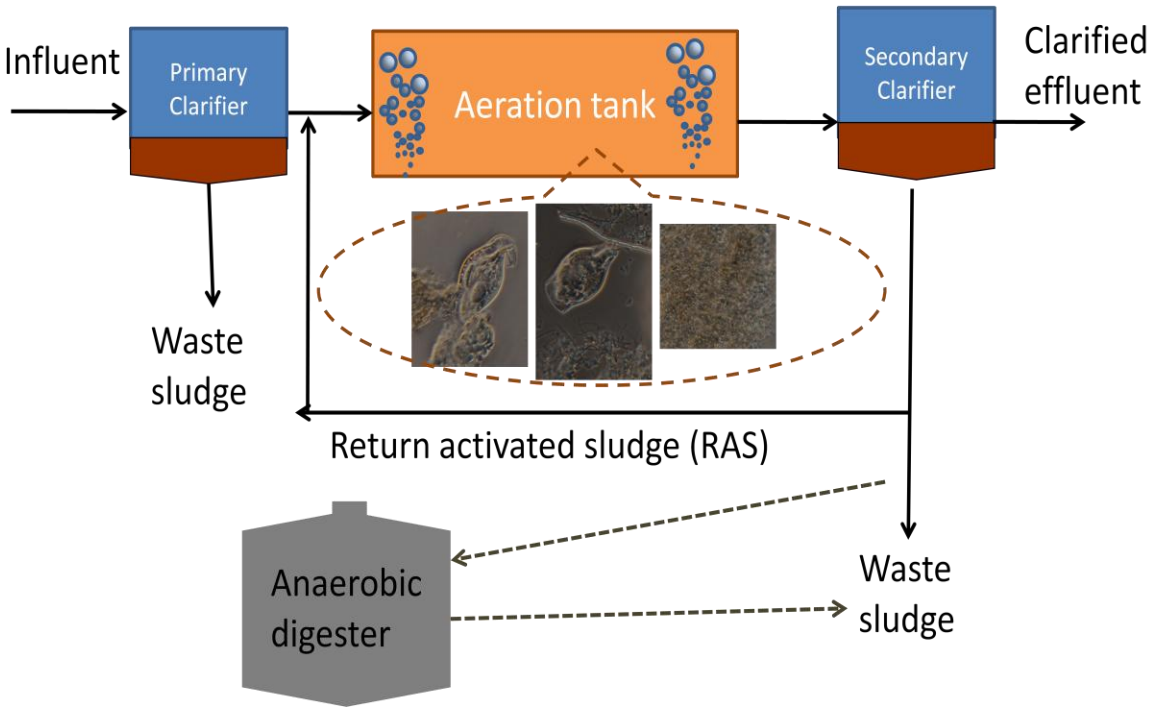


Figure 3.1 Schematics of the activated sludge process

increasing WWTPs and wastewater generation have elevated excess sludge production (Chu et al. 2009b; Park et al. 2003).

Anaerobic digestion of AS is commonly practiced to reduce the sludge volume and recover biogas, a form of renewable energy. Four phases via four categories of microorganisms are involved in digestion: (1) hydrolysis by hydrolytic bacteria, (2) acidogenesis by acidogenic bacteria, (3) acetogenesis by acetogenic bacteria, and (4) methanogenesis by methanogens (Bitton 2005). Among these stages, hydrolysis is the slow step that impedes the efficiency of digestion (Li and Noike 1992; Speece 1983). Elliot and Mahmood (2007) suggested that only half of the fed wasted activated sludge (WAS) was susceptible to anaerobic biodegradation and utilized in biogas formation; the rest of WAS existed as inorganic carbon or slowly digestible

organics. The bottleneck of hydrolysis is partly attributed to the protective cell membrane of the biomass that limits access to cell materials within, thus prompting extensive research into various pretreatments that lyse cells in order to expose the biochemical feedstock (Carrere et al. 2010). The importance of solids retention time is a result of the four processing stages cited above. The SRT in the anaerobic digester should be equal to or greater than the required minimum SRT, or the digestion process will fail eventually because of the limiting bacteria growth rate (Metcalf and Eddy Inc. 2004). Depending on the operating temperature, digester type, and desired solids destruction rate, the design SRT varied from 10 to over 30 days (Metcalf and Eddy Inc. 2004). Expected benefits of sludge pretreatment are a shorter digestion period, greater solids reduction, and increased energy production.

3.2 Ozonation of sludge for enhanced digestion

To enhance anaerobic digestion, several physical and chemical treatments have been investigated, including ultrasound, heat, vacuum, mechanical means, caustics, and ozone (Bougrier et al. 2006; Chu et al. 2008; Dewil et al. 2006; Elliott and Mahmood 2007; Tiehm et al. 2001; Vlyssides and Karlis 2004; Weemaes et al. 2000b). Various locations for treatment of sludge prior to digestion have been proposed with different objectives (Carrere et al. 2010; Chu et al. 2009b; Elliott and Mahmood 2007). Among various physical and chemical treatments, ozonation of sludge has shown promise in improving sludge treatability, available soluble COD, and biogas production (see Tables 3.1 and 3.2). Although ozonation treatment of AS has been tested in scales ranging from bench top to full-scale wastewater treatment plant, only a few studies focused on the use of low dosage for sludge ozonation (Chu et al. 2009a), such as with 23 μg O_3 g^{-1} VSS (Caravelli et al. 2006) and 13.6 mg O_3 g^{-1}

Table 3.1 Effectiveness of sludge solubilization reported in the literature

Initial sludge characteristics (mg L^{-1})	Supplied O_3 dose (mg O_3 g^{-1} TSS)	Operation time (min)	COD solub.[a] (%)	TSS solub.[a] (%)	Reference
TSS = 4600	5-20 via microbubbles	15	Not available	2.5-30	(Chu et al. 2009a)
TSS = 3500-5000 sCOD = 60-100	30-169 via microbubbles	18-100	1-26[b]	25-45	(Chu et al. 2008)
TS = 3100-3500 sCOD = 190-210	50	105	43	49	(Zhang et al. 2009)
TSS = 3100 sCOD = 20	10.8	6	5	30	(Dogruel et al. 2007)
tCOD = 7500 sCOD = 100	20-50 mg O_3 g^{-1} COD	48-55	25-31	35-65	(Weemaes et al. 2000b)
tCOD = 18000 sCOD = 700	10-180 mg O_3 g^{-1} TS (O_3 consumed)	1-45	1-44	Not Available	(Bougrier et al. 2007)
tCOD = 13000 sCOD = 100	20	Not available	8	12[c]	(Yeom et al. 2002)

[a] solubilization
[b] sCOD fraction in sludge (%)
[c] TSS solubilization = [1-(microparticles fraction + residual fraction)]

Table 3.2 Reported anaerobic digestion results with ozone-treated sludge (Carrere et al. 2010)

Substrate	Ozone dose	Anaerobic digestion conditions	Results	Reference
Mixed sludge	50 mg O_3 g^{-1} COD	Batch, 30 days, 33°C	Methane production + 36%[a]	(Weemaes et al. 2000b)
	100 mg O_3 g^{-1} COD		Methane production + 100%[a]	
Activated sludge (Synthetic)	50 mg O_3 g-1 TS	CSTR, HRT 28 days, 35°C	TS removal + 90%[a]	(Goel et al. 2003)
Activated sludge	60 mg O_3 g^{-1} TS	Batch, 18 days, 35°C	Biogas production + 50%	(Bougrier et al. 2007)
	150 mg O_3 g^{-1} TS		Biogas production + 145%	
Activated sludge	100 mg O_3 g^{-1} TS	Batch, 30 days, 36°C	Methane production + 25%[a]	(Erden and Filibeli 2011)

[a] Comparing to the anaerobic digestion performance of untreated sludge

COD of sludge (Dziurla et al. 2005). The dose of 50 mg O_3 g^{-1} dry solids was generally found optimal (Park et al. 2003; Zhang et al. 2009). Chu et al. (2009b) recently reviewed ozonation treatment for minimizing sludge production and concluded that ozone doses of 30 to 50 mg O_3 g^{-1} total suspended solids (TSS) would be technically and economically viable. However, according to life cycle assessment (LCA) of anaerobic digestion, ozonation treatment is not recommended for mixtures of kitchen waste and sewage sludge because of cost consideration. Without the optimal

ozonation conditions (e.g., ozone dose, ozone utilization rate, and operation time), ozone treatment may incur more energy usage and environmental burden than the benefits realized (Carballa et al. 2011).

Besides environmental conditions of the anaerobic digester, digestion results are influenced by process parameters such as the F/I ratio (based on either solids or COD mass ratio), liquid-to-inoculums volume ratio, and inoculum acclimation. Tomei et al. (2008) reported that the rate constant of hydrolysis was increased by one order of magnitude when the F/I ratio was increased from 0.1 to 2. They showed that when the F/I was < 1, the effect of ultrasonic treatment on digestion was not appreciable. In addition, when F/I ratio was 2 (based on VS), a minimum inoculum volume of 50% was needed to avoid interferences due to biomass limitation (Jensen et al. 2011). In contrast to unacclimated inocula, acclimated inocula with recirculation increased by 4-8% in oleic acid degradation (Pereira et al. 2001). With unacclimated inocula, an inconsistent relationship between COD solubilization and biogas production was observed and attributed to refractory compounds produced by pretreatments (Ma et al., 2011).

3.3 Pressure-assisted aeration and pressure-assisted ozonation (PAO)

High pressure gradient ($\Delta P > 30$ bar) was incorporated for cell rupture and sludge treatment (Carrere et al. 2010; Elliott and Mahmood 2007; Foster et al. 1962; Rai and Rao 2009). Volatile solids removal was increased from 35% to 50% when the AS was treated by plate collision method ($\Delta P = 30$ bar); biogas production was increased by 18% when sludge was treated by a homogenizer with 600 bars of pressure gradient (Barjenbruch and Kopplow 2003; Carrere et al. 2010; Choi et al.

1997). In applying low pressure gradient of 10 bar with CO_2, Ma et al. (2001) found only 5% COD solubilization and cumulative biogas production of 0.35 to 0.52 L g^{-1} COD removed.

Pressure-assisted ozonation (PAO) system was used for disrupting sediments and soils for the purpose of exposing contaminants, and it was found that sediment aggregates were disrupted by pressure cycles, resulting in constituent fine-grained particles with improved contaminant degradation (Hong and Nakra 2009; Hong et al. 2008b). Chu et al. (2008) employed mechanically generated ozone microbubbles to improve the effectiveness of AS treatment, and achieved high utilization of ozone, inactivation of microbes, and release of COD and other nutrients. However, when the mechanical microbubble ozonation system was used with less than 10 mg O_3 g^{-1} TSS, low concentration of intracellular materials and TSS solubilization efficiency were observed (Chu et al. 2009b).

CHAPTER 4

MATERIALS AND METHODS

This chapter presents the details of procedures and chemical/physical analyses for testing the research hypothesis. The order of presentation follows the order of results and discussion presented in Chapter 5.

4.1 Cell solubilization

4.1.1 Activated sludge

Samples of mixed liquor suspended sludge (MLSS) and returned AS (RAS) were collected weekly from the Central Valley Water Reclamation Facility (CVWRF), Salt Lake City, Utah. The sludge sample was kept in a 4-L bottle in the refrigerator (4 ^{o}C) until use within 48 h.

4.1.2 Pressure-assisted ozonation (PAO) vs. ozonation at ambient pressure

For PAO experiments, 0.3 L of MLSS or 1.2 L of RAS was placed in a 1.5-L closed reactor and subjected to compression and decompression cycles using an ozone-air mixture as detailed in a previous study (Hong et al. 2008a). The reactor was equipped with a magnetically coupled stirrer, pressure gauge, and gas vent at the reactor top and a gas inlet at the reactor bottom (Fig. 4.1). Ozone was generated

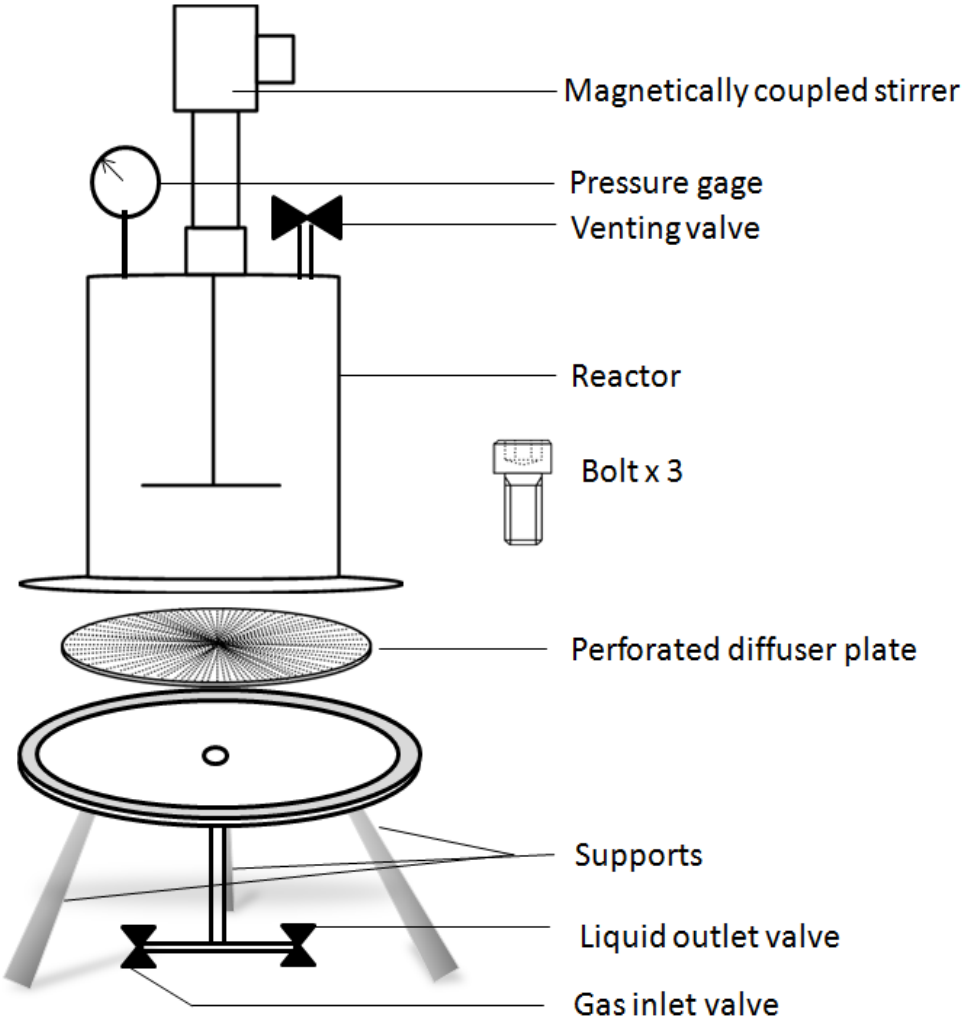


Fig. 4.1 Schematic diagram of the reactor for treatment of activated sludge

by an ozone generator (Model T-816, Polymetrics) using dry, filtered oxygen or compressed air at 105 V at the flow rate of 2 L min^{-1}. The ozone-air mixture was amended with ambient air and fed into the reactor through the inlet by use of a compressor (RIDGID model 45150). The gas stream ozone concentrations before and after the compressor (i.e., after dilution by air) were 1.5 and 0.06% (v/v), respectively, as determined by an Indigo colorimetric method (APHA et al. 2005). The supplied ozone doses of PAO pretreatments were calculated by the following equation:

$$\text{Supplied ozone doses (mg } O_3 \text{ g}^{-1} \text{ TSS)} = \left(\frac{48000}{24.4}\right)\frac{C_{ozone}V_{head}NP}{V_{sample}C_{TSS}} \quad (1)$$

where C_{ozone} = Ozone concentration (0.09% v/v) determined by Indigo blue method; V_{head} = Volume of head space in the reactor (0.3 L); N = Numbers of pressure-assisted ozonation cycle; P = Pressure (10 atm); V_{sample} = Volume of AS sample in the reactor (1.2 L); C_{TSS} = TSS concentration of sample before treatment (g L^{-1}).

Figure 4.2 illustrates the reactor and operation in pressure cycles; each pressure cycle began with the compression stage when the inlet valve was opened to allow the compressor- driven gas (i.e., ozone-air mixture) into the reactor that went through a diffuser plate at the reactor bottom, which increased the reactor headspace pressure until the designed pressure (e.g., 1040 kPa) was reached. The gas inlet valve was closed and the mixture was continually stirred for equilibration until 30 s elapsed from the end of compression; then the headspace pressure was vented through the reactor top. Foam and light particulate matter were at times observed near the water surface, and if ejected during venting, they were collected for analysis and mass balance calculation. Decompression (venting) was carried out at a controlled rate between 3 to 10 s. For treatment comparison, RAS (1.2 L) was placed in the same reactor and supplied with an ozone/air mixture (0.9% O_3 v/v) at 2 L min^{-1} under ambient pressure for a similar contact time (15 min) with continuous stirring. After ozonation, the treated sample was stirred for an additional 3 min. Ejected foam and light particulate matters were likewise collected for analysis and mass balance calculation. All treatments were replicated two or more times and

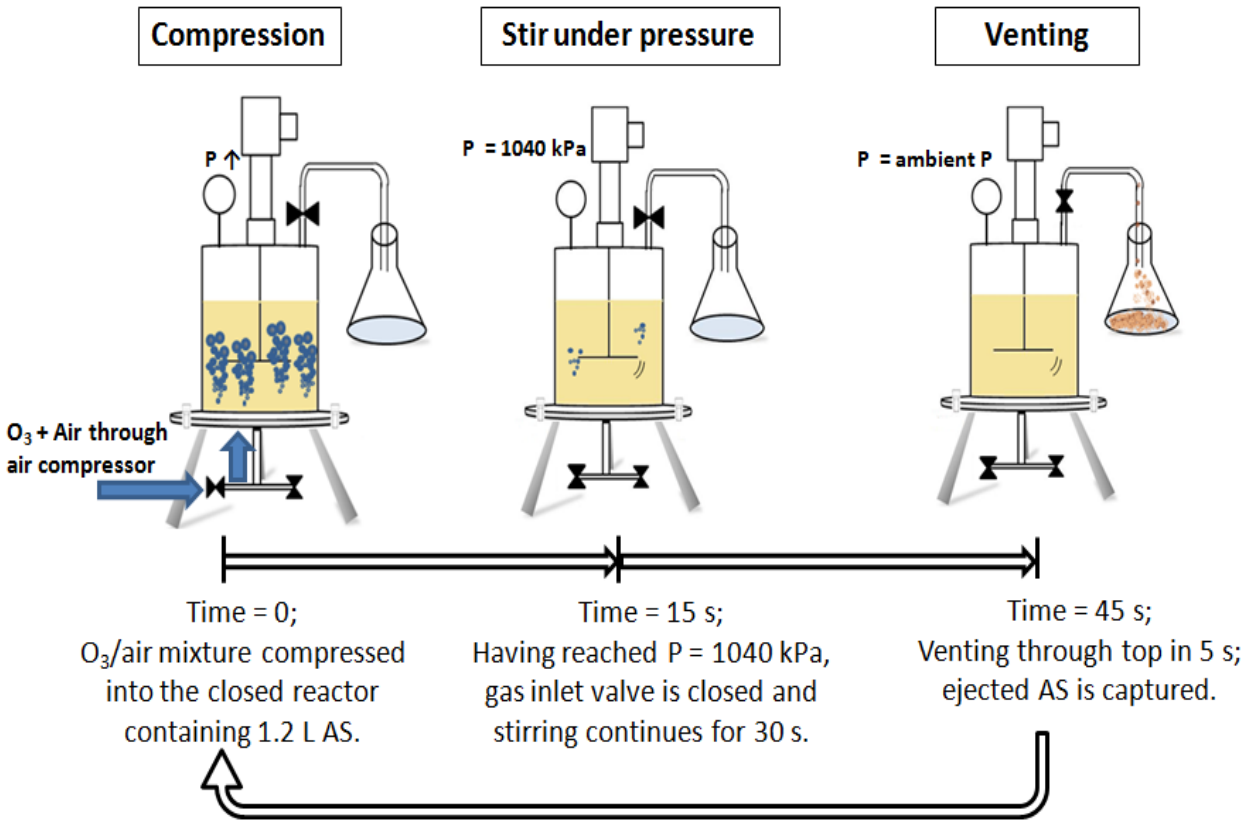


Fig. 4.2 Schematic diagram illustrating the treatment operation via pressure cycles

average results and standard deviations were shown in tables. The headspace ozone concentration ($C_{O3,h}$) was measured to calculate its consumption by RAS under different treatment conditions. While $C_{O3,h}$ under pressure was difficult to measure, it was measured immediately after decompression by withdrawing a gas volume from the headspace using a gas-tight syringe preloaded with reagents of the Indigo method (APHA et al. 2005).

4.1.3 Sludge analysis

The total and soluble COD of AS were measured (HACH COD kit and method 8000) before and after filtration through a 1.5-μm glass filter (Whatman 934-AH). To accelerate filtration, the AS was centrifuged at 1000 *g* at 20 °C for 15 min prior to filtration. The total solids (TS), volatile solids (VS), TSS, total dissolved solids (TDS), VSS, and volatile dissolved solids (VDS) were determined individually per Standard Methods 2540 (APHA, 2005). It should be noted that in this work, we define sCOD, TDS, and VDS as those in the filtrate that passed through the 1.5-μm filter. The COD and TSS solubilization efficiencies were calculated by the following equations:

$$\text{COD solubilization efficiency} = \frac{sCOD_{treated} - sCOD_{initial}}{tCOD_{initial}} \quad (2)$$

$$\text{TSS solubilization efficiency} = \frac{TSS_{initial} - TSS_{treated}}{TSS_{initial}} \quad (3)$$

4.1.4 Soluble nutrients, protein, and DNA measurements

Ozonation was either carried out by bubbling at ambient pressure for 15 min or by 20 pressure cycles up to 1040 kPa completed in 16 min. The filtrates of untreated and treated AS were determined for soluble total nitrogen (TN) (persulfate digestion; HACH method 10071), soluble NH_4 (salicylate method; HACH method 10031), nitrate (chromotropic acid method; HACH method 10020), nitrite (diazotization method; HACH method 10019), soluble total phosphorous (TP) (molybdovandate method with acid persulfate digestion; HACH method 10127), and

protein concentrations. Modified Bradford protein assay (Bio-Rad Protein Assay Kit) was performed. Each bovine serum albumin standard (20 μL) or sample solution (100 μL) was added into a clean test tube with 1 mL of diluted dye reagent (1 part of dye reagent with 4 parts of distilled-deionized water). The tube was incubated at room temperature (20 ± 2 ^{o}C) for 5 min and the content's absorbance measured at 595 nm. DNA fragments before and after treatment were analyzed by DNA electrophoresis (40 μL per sample) (VWR Midi Plus 15 Horizontal Electrophoresis Systems) running at 150 V for about 30 min. Duplicate DNA concentrations in treated RAS were determined by reaction involving diphenylamine (Johnson 1994). Due to different batches of AS with different solids concentrations sampled over time that could impact ozone dose (i.e., mg O_3 g^{-1} VSS) and treatment effectiveness, the N, P, DNA contents, and solids reduction were determined for the specific batches.

4.2 Anaerobic digestion of treated activated sludge

4.2.1 Activated sludge and anaerobic sludge inoculum

Weekly samples of returned activated sludge (RAS) were taken from CVWRF, Salt Lake City, Utah. The RAS sample was kept in a 4-L bottle in the refrigerator (4 ^{o}C) until use within 48 h. Two inoculums were used for the study; one was obtained at the anaerobic digester of the CVWRF and used on the same day while the other was from the same source but it had been incubated in two 2-L jacketed beakers at 35^{o}C, being fed regularly with RAS and with both SRT and HRT for 21 days. The inoculum from CVWRF was of 12-14 g VS L^{-1} and VSS/TSS ratio of 0.7; the incubated inoculum was of 3-5 g VSS L^{-1} and VSS/TSS ratio of 0.8.

4.2.2 Treatment of the activated sludge feed

In PAO treatment, 1.2 L of RAS was placed in a 1.5-L closed reactor and subjected to 10 or 20 cycles of compression and decompression, as described in Section 4.1.2. Each pressure cycle began by compressing an ozone-air mixture into the reactor to reach 1040 kPa; once reaching the target pressure, it was held there for 30 s for equilibration and then quickly vented to the ambient pressure for 3-5 s. Using conventional ozonation for comparison, the same volume of RAS was placed in the same reactor and contacted with an ozone/air mixture (0.9% O_3 v/v) bubbling through the suspension at 2 L min^{-1} for 15 min under ambient pressure. In all ozonation treatments, any ejected foam and light particulate matters were collected for analysis and mass balance calculation.

4.2.3 Feed characteristics

All measurements of AS solids, COD, and sludge feed including untreated and treated RAS were replicated two or more times and average results and standard deviations were shown in Table 4.1 and Table 4.2. The tCOD and sCOD of untreated and treated RAS were determined by closed reflux colorimetric method (APHA et al. 2005) before and after filtration through a 1.5-μm glass filter (Whatman 934-AH). The total solids (TS), volatile solids (VS), TSS, and VSS were determined individually per Standard Methods 2540 (APHA et al. 2005). The COD and TSS solubilization efficiencies were defined in Section 4.1.3.

Table 4.1 Solids characteristics (mg L^{-1}) of activated sludge without and with ozonation treatment prior to anaerobic digestion with inoculums from CVWRF

	Ozone dose (mg O_3 g^{-1} TSS)	TSS	VSS	COD	sCOD	pH	COD solubilization (%)
No treatment	-	8543 ± 152	6997 ± 109	12563 ± 3834	31 ± 3	7.5-7.8	-
10 cycles PAO	5	7904 ± 560	6429 ± 439	13298 ± 1785	451 ± 72	6.5-6.9	4
20 cycles PAO	10	7567 ± 141	6098 ± 98	11950 ± 552	1612 ± 84	6.7-6.8	15
15 min conventional ozonation	52	8127 ± 3	6606 ± 65	11160 ± 123	689 ± 123	6.7-6.8	6

Table4.2. Characteristics (mg L^{-1}) of ozonated activated sludge prior to anaerobic digestion

	Ozone dose (mg O_3 g^{-1} TSS)	TSS	VSS	COD	sCOD	pH	COD solubilization (%)
No treatment	-	6138 ± 111	5054 ± 105	7855 ± 1068	40 ± 21	7.3-7.7	-
20 cycles PAO	10	4869 ± 298	3902 ± 208	7625 ± 219	1588 ± 378	6.2-6.8	20
15 min conventional ozonation	52	4934 ± 1012	4390 ± 242	7240 ± 679	846 ± 7	6.9-7.1	10

4.2.4 Anaerobic digestion

4.2.4.1 Anaerobic digestion with inoculum immediately obtained from CVWRF

Digestion was carried out over 14 days in anaerobic batches of 125-mL Erlenmeyer flasks; the flasks were prepared by nitrogen purging and mixing the AS with inoculum in different amounts to have a 49-68 mL of working volume, resulting in different F/I ratios at 0.5, 1, and 2 g VS/g VS. All flasks were sealed by a sleeve-type rubber stopper and were incubated in a water bath shaker (New Brunswick G76). Each flask was connected to a DI water-filled gas collection tube, which measured specific biogas production (mL biogas g^{-1} COD_{added}) via liquid movement, as shown in Fig. 4.3. The COD, solids, and pH of the digested RAS/inoculum mixture were measured in duplicate at regular time intervals within the 14-days digestion test period per Standard Methods (APHA, 2005).

4.2.4.2 Anaerobic digestion with inoculum prior acclimated in the laboratory

Digestion was carried out over 20 days in anaerobic batches in the same manner as aforementioned using laboratory-acclimated inoculums, using an inoculum volume of 30 mL with different sludge volume to achieve the F/M ratios of 0.5 or 1 g COD_{AS} g^{-1} $VSS_{inoculum}$ (F/I = 0.4 or 0.8 g VS_{AS}/g $VS_{inoculum}$). The treated and untreated (20 PAO cycles) RAS samples (3-22 mL) were placed into individual flasks in duplicate or more according to the test F/M ratios, and were incubated for 20 days. The COD, solids, and pH of the digested RAS/inoculum mixtures were measured in triplicate or more at day 0 and 20 per Standard Methods (APHA et al. 2005). Biogas production was measured at regular time intervals within the 20 days of digestion.

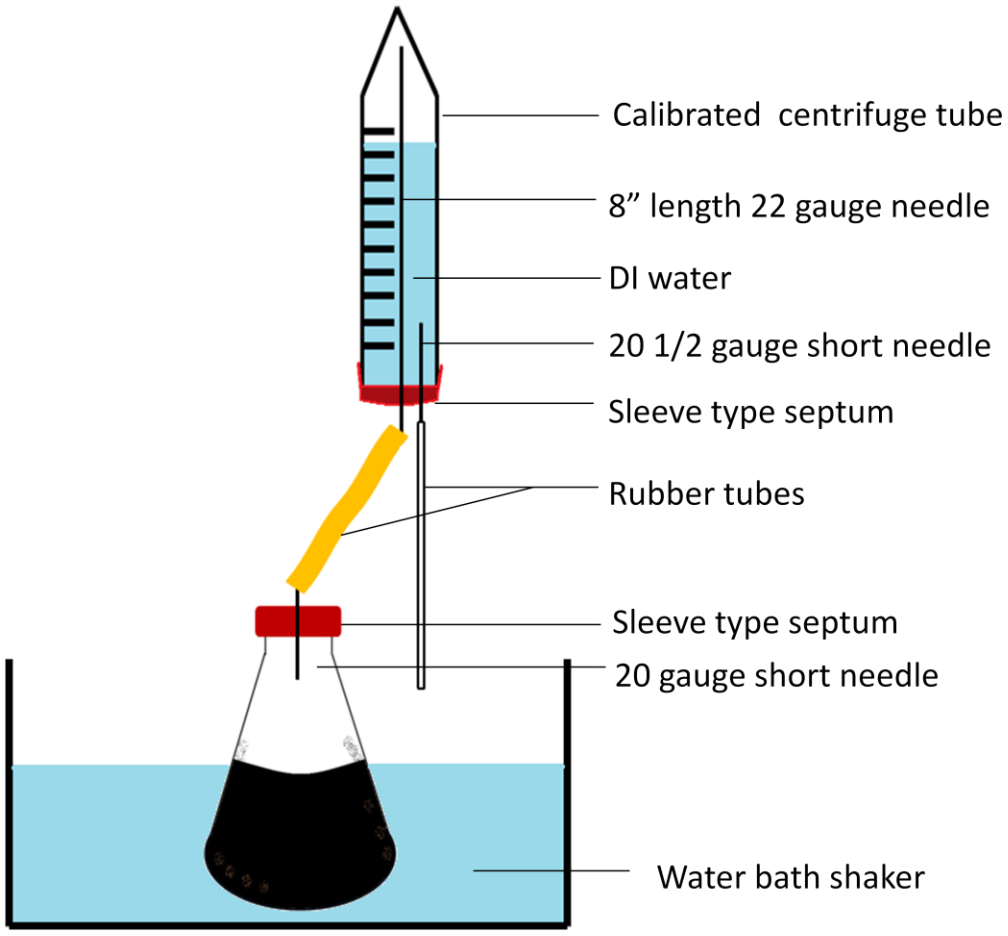


Figure 4.3 Schematic of digestion flask and biogas collection

CHAPTER 5

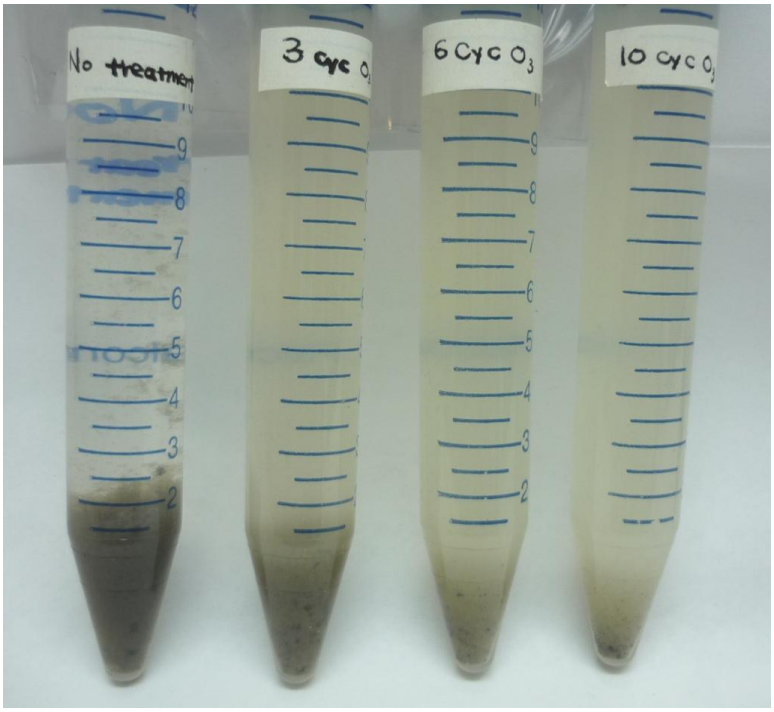
RESULTS AND DISCUSSION

5.1 Cell solubilization

5.1.1 Cell solubilization of MLSS

The mixed-liquor suspended solids (MLSS) (300 mL) were treated with 0, 3, 6, and 10 pressure cycles at elevated pressure of 1040 kPa. Photographs (Fig. 5.1) show less settled solids and more yellowish supernatants after pressure-assisted ozonation, which are consistent with more soluble materials or intracellular materials released from the flocs after pressure-assisted ozonation (Fig. 5.1). Figure 5.1 also shows increasing soluble COD and dissolved solids with an increasing number of pressure cycles. After 3, 6, and 10 cycles of pressure-assisted ozonation, the suspend solids concentration decreased with the increasing cycles. In addition, more soluble materials were obtained when the supplied ozone was increased from 0.05 to 0.15 g O_3 g^{-1} TSS while the treatment pressure was kept the same.

Separate DNA analyses conducted on AS that had been subjected to 3 pressure cycles of ozonation plus 7 cycles of aeration (1040 kPa) found a significant presence of DNA showing DNA smears in gel electrophoresis results. DNA was increased from absence before treatment to 6 mg L^{-1} after treatment as measured by the diphenylamine method (Table 5.1).



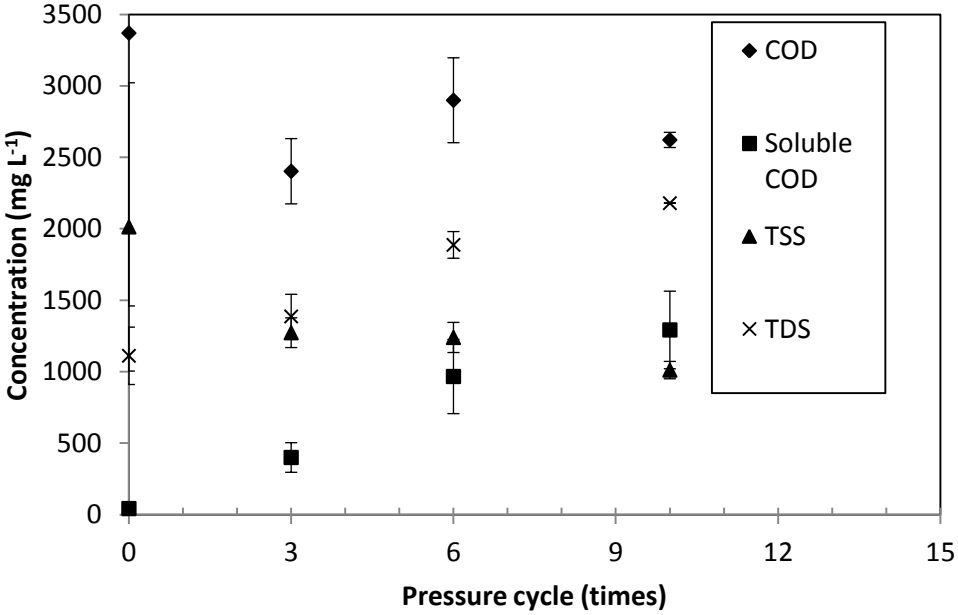


Figure 5.1 COD and settled solids photograph (upper) and characteristics (lower) of untreated and after treatment of the MLSS by various ozonation pressure cycles at 1040 kPa (150 psi)

Table 5.1 DNA and protein concentrations (mg L^{-1}) of returned activated sludge before and after 3 ozonation and 7 aeration pressure cycles (P = 1040 kPa) (results in duplicate)

Treatment	No treatment	3 ozonation plus 7 aeration cycles
DNA	6±3	35±3
Protein	Not present	41±4

Figure 5.2 shows AS prior to treatment (upper pictures) and after 3 ozonation cycles (with 0.06% O_3) followed by 7 aeration cycles (with air only) reaching 1040 kPa (lower pictures). Prior to treatment, the microscopic picture shows an abundance of filamentous bacteria and flocs with a discernable rotifer present. After treatment, it shows little biomass remaining but disperse nondescript flocs and viscous patches of liquid in the water. Apparently, disintegration of the AS occurred during 10 pressure cycles of ozonation and aeration.

Table 5.2 shows the total and soluble COD (tCOD and sCOD) measured for the MLSS before and after various treatments. Several studies showed similar results that the tCOD decreased after treatment of activated sludge with ozone at ambient pressure (Chu et al. 2008; Dogruel et al. 2007; Weemaes et al. 2000b), which was indicative of decreasing tCOD in the flocs brought on by ozone oxidation. Moreover, the increased sCOD of treated sludge shows that because of pressure-assisted ozonation and aeration, particulates have been transformed into a soluble fraction which is readily digested by bacteria (Bougrier et al. 2006). In addition, the

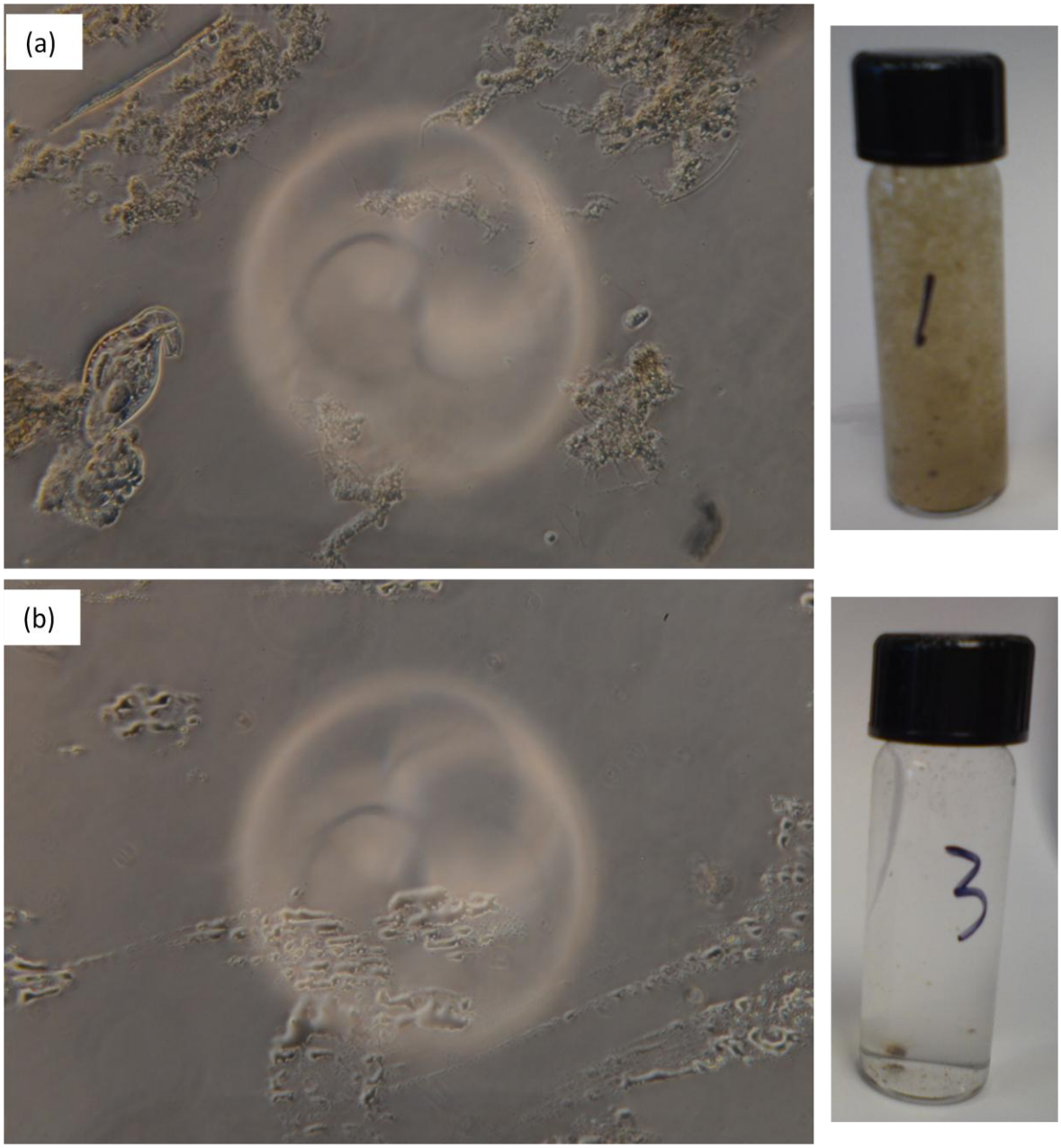


Figure 5.2 Photographs and microscopic pictures of activated sludge (MLSS) via phase contrast microscopy at (a) 200X magnification before treatment, and (b) 800X magnification after treatment by 3 ozonation plus 7 aeration cycles to pressure of 1040 kPa with rapid venting (3 s)

Table 5.2 Characteristics of MLSS before and after various treatments (AS volume of 300 mL)

	Compression, equilibration time (s)	Vent time (s)	tCOD	sCOD	sCOD/tCOD
MLSS					
None[a]			4905 ±920	36 ±8	0.007
1+ 9 1040 kPa[b]	30, 30	150	3900 ±354	142 ±27	0.036
3+7 350 kPa[b]	5, 55	40	3385 ±318	158 ±16	0.047
3+7 690 kPa[b]	15, 45	60	2905 ±226	353 ±2	0.122
3+7 1040 kPa[b]	30, 30	150	3658 ±1248	600 ±57	0.164

[a] Solids analysis was replicated 7 times, BOD_5 measurement was replicated 4 times.
[b] Duplicates
[c] N.A.: Data not available

sCOD/tCOD ratio indicates the relative amount of soluble contents that can be utilized and this ratio increases from 0.007 to 0.16, an increase by 23-fold after the said treatment.

Figure 5.3 shows the fractions of various solids in the 300 mL of MLSS after different treatments. After ozonation/aeration treatment, the total solids concentration with varied treatment conditions decreased from 4800 to 1800-3600 mg L^{-1}. The diminished TS in the treated suspension may be due to a portion of the

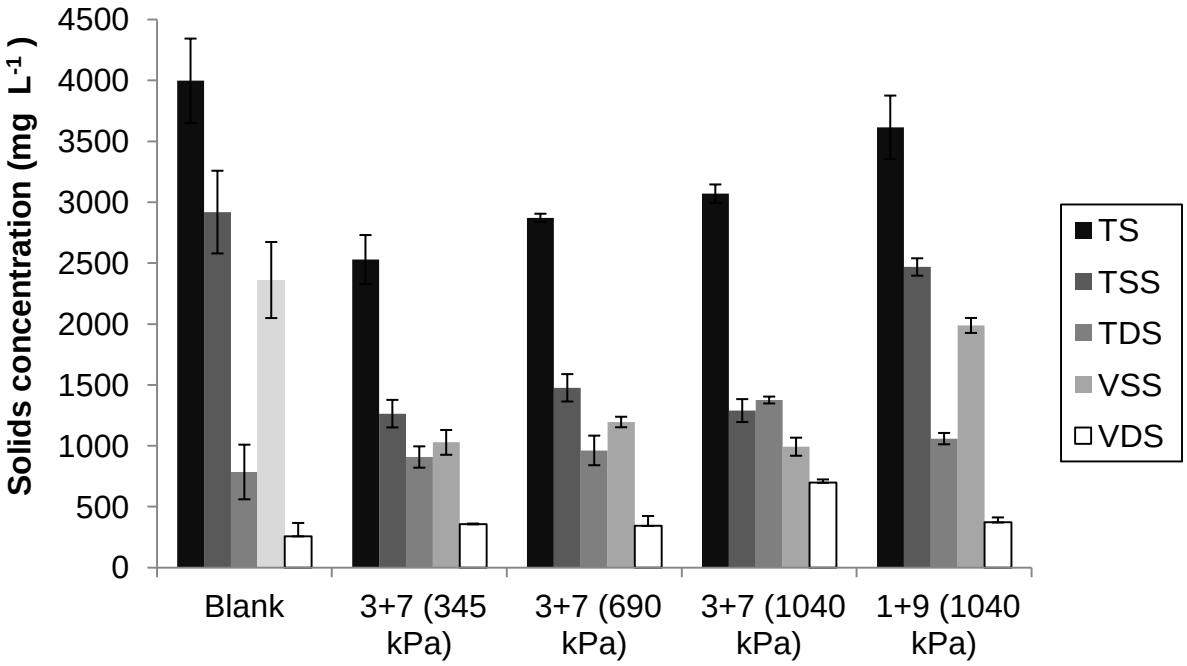


Figure 5.3 Solids characteristics of untreated and treated activated sludge (MLSS) according to pressures (350, 690, and 1040 kPa) and various ozonation plus aeration cycles. Operation conditions were shown in the MLSS section of Table 5.2

"cell water content" being homogeneously incorporated into the bulk water phase when the microbes were ruptured and ozone oxidation occurred (Chu et al. 2008; Manterola et al. 2008; Zhang et al. 2009). The TDS/TSS ratios in MLSS following various treatments increased from 0.27 before treatment to 1.1 after, while the VDS/TSS ratios showed corresponding increases from 0.088 to 0.54. The VDS content is expected to reflect the amount of solubilized cell substances after rupture. The increased VDS in the treated activated sludge showed that the PAO plus aeration method may improve the digestibility of activated sludge for aerobic or anaerobic bacteria.

5.1.2 Cell solubilization of RAS

Figure 5.4 and Table 5.3 show the solids characteristics and pH of sludge after various ozonation treatments. Changes of TS after ozonation at ambient pressure or via pressure cycles were not significant at p = 0.05 level, while TSS were significantly reduced after ozonation. After treatment with 77 mg O_3 g^{-1} TSS at ambient pressure, TSS decreased by 12% and TDS increased by 160%. After treatment, the TSS solubilization efficiency was determined to be 12% and sCOD increased by 1600% from 50 to 796 mg L^{-1}. When ozonation was carried out in pressure cycles at 4.8 to 11 mg O_3 g^{-1} TSS over 8-16 min, TSS reduced by 15-25% corresponding to an increase of TDS by 140-220%. Among various treatments with pressure cycles shown in Fig. 5.4, the least changes in TSS (decrease) and TDS (increase) were seen with 15 pressure cycles at 690 kPa in contrast to the maximum changes of TSS and TDS with 20 pressure cycles at 1040 kPa. When the compression pressure and the number of pressure cycles were increased, COD and TSS solubilization efficiencies of the AS increased by 7-20 and 18-25%, respectively. In the experimental series with compression pressure of 1040 kPa, more changes in TSS and TDS were seen with an increasing number of pressure cycles. The measured TS agreed with the combined amounts of individually measured TSS and TDS well within 3%, indicating a good mass balance of the measured values (Fig. 5.4). Ozonation using pressure cycles was compared to that under ambient pressure. While the amount of ozone supplied during 16 min of pressure cycles was 10-15 times less than that of ozonation at ambient pressure in the same period, similar or higher COD and TSS solubilization were obtained with pressure cycles (Table 5.3). Throughout this work, relative standard deviations of measured TS and tCOD

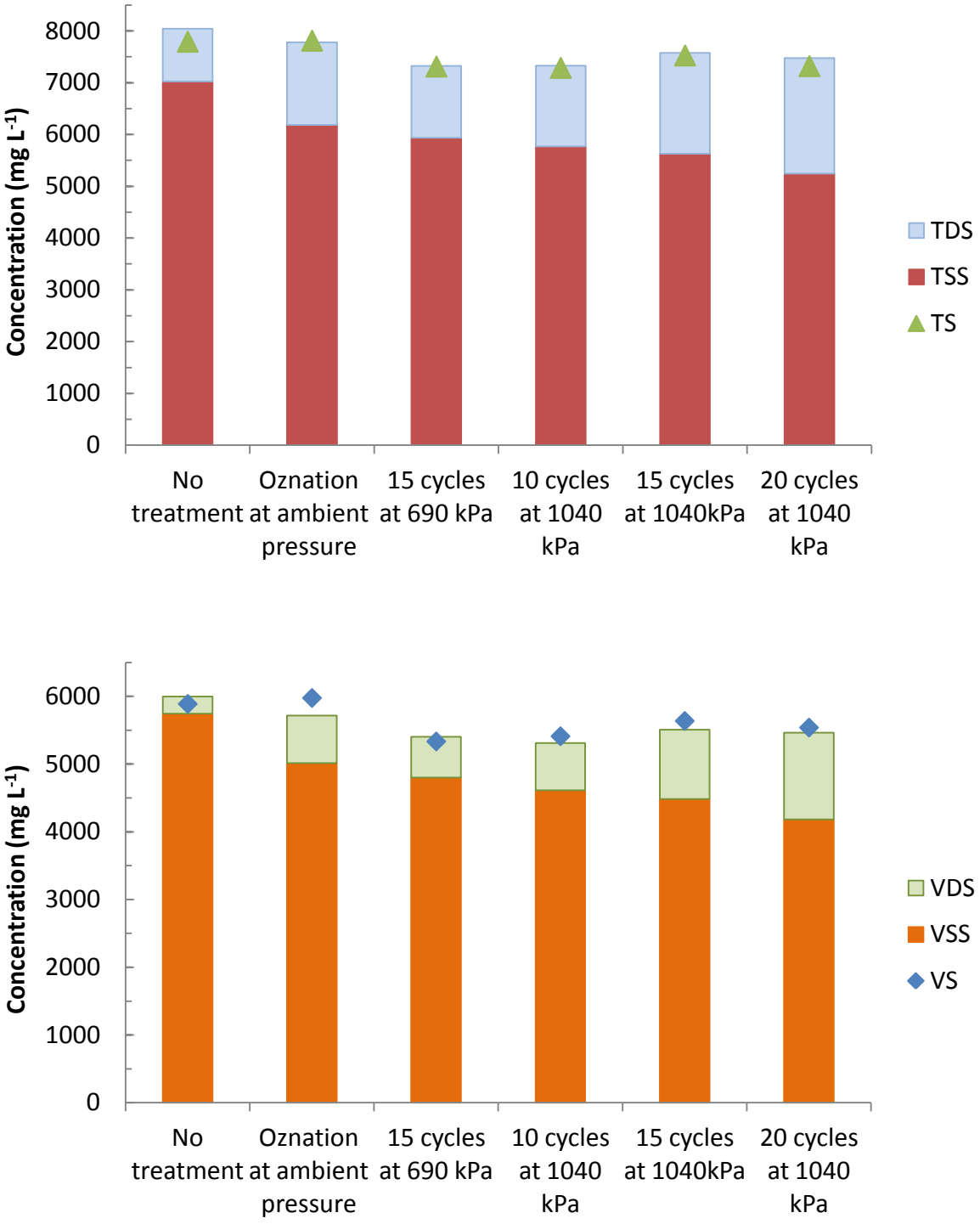


Figure 5.4 AS solids properties (TS, TSS, TDS, VS, VSS, and VDS) before and after treatment. Supplied ozone doses (mg O_3 g^{-1} TSS) shown in Table 5.3

Table 5.3 Change in tCOD and pH before and after various treatments, with COD and TSS solubilization efficiencies determined

Treatment	Ozone supplied (mg O_3 g^{-1} TSS)	tCOD (mg L^{-1})	COD solubilization %	TSS solubilization %	pH
No treatment	-	8200 ± 720			6.6-7.7
Ozonation at ambient pressure	77 ± 7	8500 ± 600	9	12	6.0-7.0
5 cycles at 690 kPa	5.6 ± 1	8000 ± 0	7	16	6.1-7.1
10 cycles at 1040 kPa	4.8 ± 0	7500 ± 1200	9	18	6.3-6.5
15 cycles at 1040 kPa	7.9 ± 1	8000 ± 680	16	20	6.2-6.5
20 cycles at 1040 kPa	11 ± 1	7900 ± 120	21	25	6.8-6.5

values of the treated AS were ± 5 and ± 9%, respectively. A loss of TS after ozonation was often observed and attributed to mineralization by ozone (Chu et al. 2008; Manterola et al. 2008; Zhang et al. 2009). The variation of TS and tCOD between treatments was not significant at p = 0.05 level. Additionally, calculation of mineralization of biomass, assuming as $C_5H_7NO_2$, would amount to 565 mg of biomass based on a dose of 77 mg O_3 g^{-1} TSS used for the test batch at ambient pressure and amount to 53 mg of biomass based on a dose of 11 mg O_3 g^{-1} TSS used with 20 pressure cycles; observed changes were not statistically significant. The pH decreased by about 1 unit afterward due to acidification by ozonation, as expected.

The soluble TN, NH_4, nitrate, nitrite, protein, and TP concentrations in the AS filtrate before and after ozonation were used to confirm the release of cell contents, as shown in Table 5.4. Small amounts of soluble NH_4-N (3 to 7 mg L^{-1}) were found before and after ozonation. After ozonation at ambient pressure with 87 mg O_3 g^{-1} TSS over 15 min, soluble TN increased by 710% and TP by 430% along with an increase of protein contents from initially absent to 31 mg L^{-1}. The soluble nitrate and nitrite contents of ozonated AS were increased by > 100- and 5-fold, respectively. Similar levels of increases in soluble TN, nitrate, nitrite, and soluble TP (860, 10000, 250, and 640%, respectively) along with 39 mg L^{-1} of proteins were found after ozonation in pressure cycles, albeit at a much lower dose of 12 mg O_3 g^{-1}

Table 5.4 Changes of AS in soluble TN, NH_4, nitrate, nitrite, proteins, and TP after nitrate and nitrite contents of ozonated AS were increased by > 100- and 5-fold, various treatments

Treatment	Ozone supplied (mg O_3 g^{-1} TSS)	Soluble TN (mg L^{-1})	Soluble NH_4 (mg L^{-1})	Soluble Nitrate /Nitrite (mg L^{-1})	Soluble protein (mg L^{-1})	Soluble TP (mg L^{-1})	VSS reduction %
No treatment	-	14 ± 4	7.3 ± 0	0.5/ 0.12	Not detected	10 ± 1	-
Ozonation at ambient pressure	87 ± 3	100 ± 20	6.3 ± 0.6	> 50[a]/ 0.64	31 ± 1	43 ± 6	9
20 cycles at 1040 kPa	12 ± 0	120 ± 0	2.6 ± 0.4	> 50/ 0.3	39 ± 4	64 ± 3	22

[a]Upper limit of analytical range.

TSS delivered via pressure cycles over the same period. The increases of soluble TN including the soluble nitrate and nitrite were attributed to organic N in released intracellular materials that were oxidized by ozone to NH_3 and then to nitrite and nitrate. In addition, VSS reduction was by 22% with 12 mg O_3 g^{-1} TSS using pressure cycles compared to VSS reduction by 9% with 87 mg O_3 g^{-1} TSS using ozonation at ambient pressure (Table 5.4), which hinted at more effective solubilization of AS into intracellular materials in the aqueous phase via pressure cycles.

The ozone concentration in the headspace immediately after decompression was measured with tap water and RAS in the reactor, respectively; the results showed during 20 pressure cycles reaching 1040 kPa, ozone concentration of 0.21-0.23% (v/v) was measured in the headspace over tap water (1.2 L) after the 5th, 10th, 15th, and 20th pressure cycles while there was ozone concentration of only 0.06-0.09% (v/v) in the headspace over the RAS slurry at these points of pressure cycles. The ozone concentration in the headspace over tap water and RAS in the reactor during ozonation at ambient pressure were also measured; the results showed ozone concentrations of 0.8, 0.9, and 0.9% (v/v) over tap water after 5, 10, and 15 min, respectively, and 0.3, 0.5, and 0.7% (v/v) over RAS at the same points in time, respectively. These results suggest that assuming relatively slow (i.e., negligible) hydrolysis of ozone in tap water, the ozone utilization rates by RAS were 74% at 5, 10, and 15 pressure cycles and 57% at the 20 pressure cycle, while the utilization rates were 60, 48, and 20% ozone at 5, 10, and 15 min of ozonation at ambient pressure. The amounts of ozone actually consumed by RAS via 20 pressure cycles of ozonation and via 15 min of ozonation at ambient pressure were thus estimated to be 18 and 22 mg O_3 g^{-1} TSS, respectively. While ozone consumptions in both cases

were similar, the RAS was subjected to a smaller ozone supply via pressure cycles (11 mg O_3 g^{-1} TSS) than during ambient pressure (77 mg O_3 g^{-1} TSS), yet the former treatment mode resulted in higher COD (12% more) and solids reduction (13% more), which suggested improved efficiency with pressure cycles (Table 5.3).

Frigon and Isazadeh (2011) summarized three well postulated effects of ozone on returned AS: (1) cell lysis with material release (Bougrier et al. 2007; Chu et al. 2009a; Chu et al. 2009b); (2) AS solids transformed (not oxidized) into soluble and particulate biodegradable COD (Manterola et al. 2008); and (3) sludge biomass inactivated (killed) (Chu et al. 2008). Despite differing views on ozonation actions on AS, increased soluble organic contents accompanied by reduced suspended solids were well reported (Chu et al. 2009a). In this study, increasing TDS and VDS accompanied by decreasing TSS and VSS of AS after treatment strongly suggested solubilization. Our observation of an increase of sCOD proportional to the increase of TDS or VDS following ozonation, which corresponded to an increased TDS/TSS ratio, suggested disintegration of the AS flocs and damage of cell membrane due to ozone exposure, as previously reported (Chu et al. 2009a; Zhang et al. 2009). The increases in soluble TN, NH_4, TP, and protein concentrations after ozonation (Table 5.4) again pointed to oxidation by ozone of released intracellular materials, agreeing with the literature (Chu et al. 2008; Dogruel et al. 2007; Zhang et al. 2009).

The solubilization results of PAO of AS from this work are compared to previous studies with similar ozone dosage, though not all relevant and comparable data are available. As shown in Table 5.5, various extents of solubilization resulted when AS was subjected to 5-180 mg O_3 g^{-1} TSS for 1 to > 60 min. Characteristics of AS samples in these studies varied significantly, such as in tCOD from 4000 mg L^{-1}

Table 5.5 Solubilization of AS by ozonation in various studies

Initial sludge characteristics (mg L^{-1})	Supplied O_3 dose (mg O_3 g^{-1} TSS)	Operation time (min)	Ratios of sCOD/tCOD before & after	COD solub.[a] (%)	TSS solub.[a] (%)	Reference
tCOD = 8200; sCOD = 50	11 via 20 pressure cycles (P = 1040 kPa)	16[b]	0.006 & 0.22	21	25	This work
TSS = 4600	5-20 via microbubbles	15	Not available	Not available	2.5-30	(Chu et al. 2009a)
TSS = 3500-5000; sCOD = 60-100	30-169 via microbubbles	18-100	0.025 & 0.12-0.31	1-26[c]	25-45	(Chu et al. 2008)
TS = 3100-3500; sCOD = 190-210	50	105	Not available	43	49	(Zhang et al. 2009)
TSS = 3100; sCOD = 20	10.8	6	0.007 & 0.07	5	30	(Dogruel et al. 2007)
tCOD = 7500; sCOD = 100	20-50 mg O_3 g^{-1} COD	48-55	0.01 & 0.29-0.45	25-31	35-65	(Weemaes et al. 2000b)
tCOD = 18000; sCOD = 700	10-180 mg O_3 g^{-1} TS (O_3 consumed)	1-45	0.04 & 0.05-0.5	1-44	Not Available	(Bougrier et al. 2007)
tCOD = 13000; sCOD = 100	20	Not available	0.008 & 0.091	8	12[d]	(Yeom et al. 2002)

[a] Solubilization

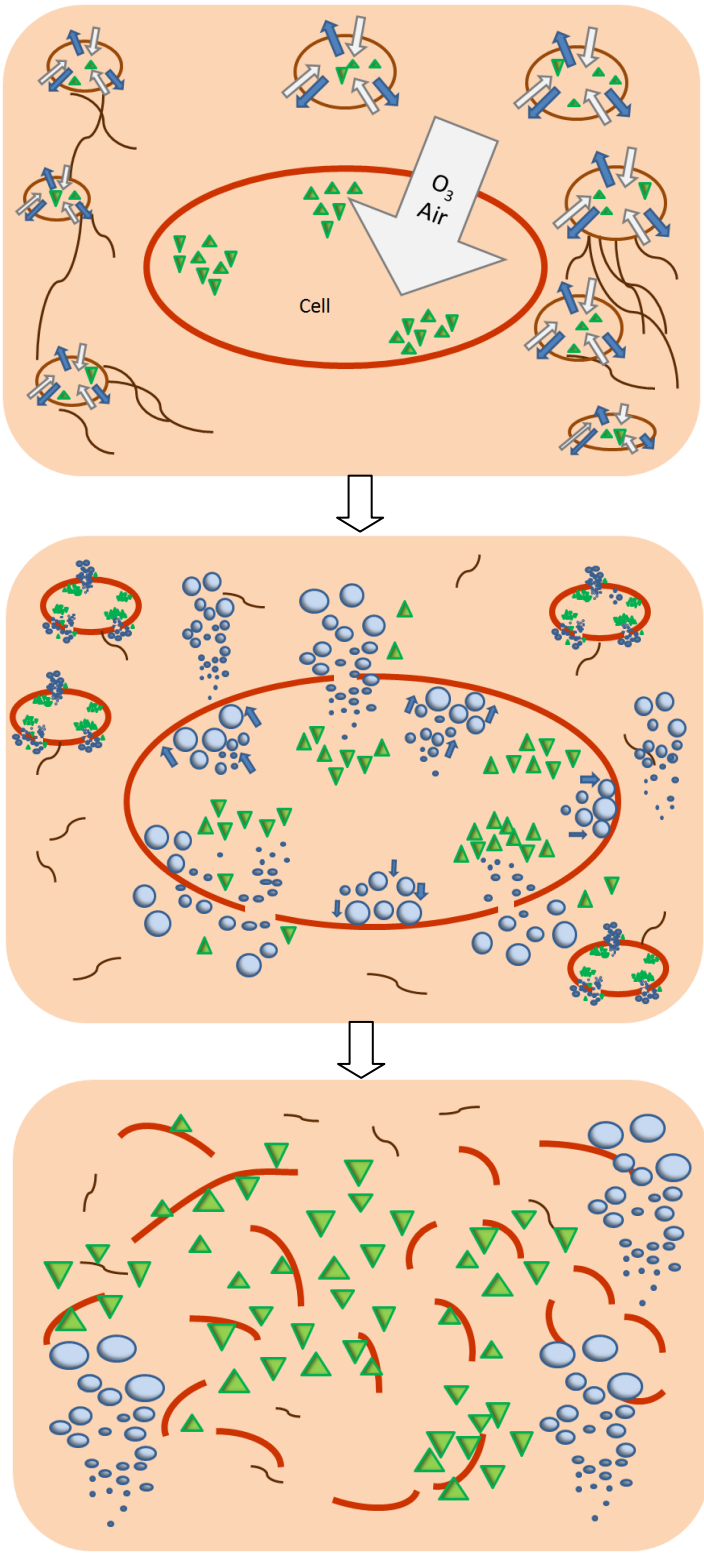
[b] Test conditions: 1.2 L of RAS in the 1.5 L reactor (20% headspace); compression to reach 1040 kPa in 15 s, equilibration time of 30 s, and vent time of 3 s

[c] sCOD fraction in sludge (%)

[d] TSS solubilization = [1-(microparticles fraction + residual fraction)]

in MLSS to > 17000 mg L^{-1} in RAS as well as in sCOD from 50 to 700 mg L^{-1} (Table 5.3). For this reason, solubilization was evaluated based on multiple parameters including sCOD/tCOD, COD solubilization (%), and TSS solubilization (%) before and after treatment. These studies showed that after treatment, the ratios of sCOD/tCOD increased significantly (1.25 to 40 times) while the COD solubilization ranged from 1 to 47% and TSS solubilization from 2.5 to 65%. In general, the values of these indicator parameters increased with increasing ozone dose (g O_3 g^{-1} TSS), signifying increased solubilization with increased ozonation (Kianmehr et al. 2010). Our present work showed a dose of 10 mg O_3 g^{-1} TSS administered on the returned AS via 20 pressure cycles of ozonation over 16 min resulted in an increase of sCOD/tCOD ratio by 37-fold (from 0.006 to 0.22), while COD solubilization and TSS solubilization were 21 and 25%, respectively. These numbers were within the reported ranges. However, it should be noted that the ozone dose and operation time employed in the present study were among the lowest showing comparable solubilization results; this was made possible by use of pressure cycles in the delivery of ozone and its unique mode of contact with AS.

Ozonation with microbubbles was found advantageous to conventional bubble contact device in terms of ozone utilization and sludge solubilization. By using microbubbles in lieu of conventional bubbling contact at the same dosage, Chu et al. (2008) found that more than 2-fold of COD and TN along with 8-fold of TP were released from the sludge into the supernatant. To explain enhanced sludge solubilization with expanding microbubbles, we proposed possible rupture mechanisms during pressure cycles, as shown in Fig. 5.5. The transport of small molecules such as O_2, CO_2, and H_2O across the membrane of microbes is routine for



(a) Transport: Upon compression, dissolved gas at elevated concentration under pressure migrates across membrane into cells.

(b) Relieved of pressure by decompression, dissolved gas at now oversaturated concentration escapes via bubbles that form and expand within cell and migrate across membrane to bulk liquid.

(c) Repeated pressure cycles with ozone cause deterioration in membrane to regulate flow of substances, accelerating toward loss of integrity.

Figure 5.5 Proposed mechanisms of AS disintegration and rupture with pressure-assisted ozonation cycles

life maintenance. However, uncontrolled transport that results in release of cell materials can be accelerated for several reasons. First, compression of gas into the reactor results in a much higher dissolved gas concentration in the bulk liquid that the microorganisms have rarely encountered against such concentration gradient, the microbes are ill equipped to defend. Second, increased molecular diffusion across the cell membrane occurs due to steepened concentration gradient, increasing the dissolved gas concentration within; when the pressure is rapidly reduced, gas formation and expansion occur within the cell, causing the formation of gas pockets in the cell (as diffusive transport across the membrane is slower than decompression). Gas accumulation in the cells during repeated pressure cycles contributes to possible rupture of the cells. Third, it is conceivable that the cell membrane exhibits elasticity in dealing with rapid pressure changes during the pressure cycles. However, ozone acts to oxidize and cause the membrane to deteriorate, losing its flexibility and the ability to regulate material flow, which leads to increased susceptibility to rupture in subsequent cycles.

Previously observed cell rupture due to rapid release of hyperbaric pressure would support the proposed mechanism involving expanding gas. Park and Clark (2002) found rupturing of cell envelop of the microbe *Methanococcus jannaschii*, a deep-sea methanogen, when it was subjected to a hyperbaric pressure of 26 MPa (260 atm) and rapid decompression (ca 1 s) and that rupturing significantly lessened with gradual decompression over 5 min. In contrast, decompression from a hydrostatic pressure of 26 MPa did not induce cell lysis. The authors attributed the observed rupture to increased intracellular solubility of helium (He) employed in the reactor system at high pressure, which upon rapid decompression, the expansion of concentrated He in the cytoplasm caused cellular disruption.

5.2 Anaerobic digestion of PAO-treated activated sludge

5.2.1 Anaerobic digestion with inoculum freshly obtained from CVWRF

5.2.1.1 Solids and COD reduction during digestion

PAO treatment (20 cycles) of RAS prior to anaerobic digestion reduced its TSS by 11% and VSS by 13% (Table 4.1). Table 5.6 shows specific solids and COD reduction rates (as % reduction per g inoculum, i.e., % g^{-1} inoculum) after 14 days of anaerobic digestion in batch according to different ozonation treatments and food-to-inoculum (F/I) ratios. Generally, the solids and COD reductions of RAS during digestion were higher for RAS with PAO treatment than RAS without PAO treatment. These results show the positive impact of PAO treatment on solids

Table 5.6 Solids and COD reduction rates (as % RAS feed reduced/g inoculum) for untreated and treated AS after 14 days of anaerobic digestion at various F/I ratios

	SRT (days)			COD (% g^{-1})			VS (% g^{-1})			VSS (% g^{-1})		
F/I	0.5	1	2	0.5	1	2	0.5	1	2	0.5	1	2
No treatment	36	23	19	4.5	6.7	12	8.7	8.7	9.0	3.4	5.5	15
10 cycles PAO	29	22	18	5.7	13	10	11	14	14	5.4	11	19
20 cycles PAO	30	23	19	5.8	11	14	9.4	14	15	4.2	11	17
F/I	0.5	1	2	0.5	1	2	0.5	1	2	0.5	1	2
No treatment	36	21	17		3.6	10		8.0	6.1		5.8	12
Ozonation at ambient pressure	31	21	17	5.3	4.5	4.7	11	14	11	5.5	12	12

reduction during anaerobic digestion. However, as only limited cases of ozonation conditions had been tested, the results did not show increasing reduction rates with more extensive ozonation treatment or enable the identification of an optimal dose. The results further show the F/I ratio as an important process parameter that impacts reduction rates. Even without ozonation treatment, COD and solids reduction rates of RAS during digestion increased with increasing F/I ratio. Given the same PAO treatment, solids and COD reduction rates also increased with increasing F/I ratio from 0.5 to 2. The positive effect of conventional ozonation using 52 mg O_3 g^{-1} TS on solids and COD reduction was seen with F/I ratio of 0.5-1; however, its effect on COD reduction with F/I = 2 was not obvious (less COD reduction than in untreated samples or similar VSS reduction as in untreated samples). This may indicate positive effect of conventional ozonation only at low F/I range (e.g., ≤ 1).

5.2.1.2 sCOD vs. time profile during digestion

Figure 5.6 shows the sCOD (mg L^{-1}) vs. time profiles for untreated and ozonated AS at F/I = 0.5, 1, and 2. Initial sCOD (Table 4.1) in the treated sludge just prior to anaerobic digestion was high (450-1600 mg L^{-1}), which confirmed effective solids and COD solubilization by ozonation treatment. In many cases, sCOD continued to increase at the beginning of digestion. These increases occurred in the first 5 days, to be followed by continual decreases to <750 mg L^{-1} during the rest of the 14-days digestion period. In cases when the initial sCOD were very high (e.g. >1300 mg L^{-1} in the case of conventional ozonation with F/I of 1, or in cases of 10 and 20 cycles of PAO with F/I =2), sCOD decreased immediately from the

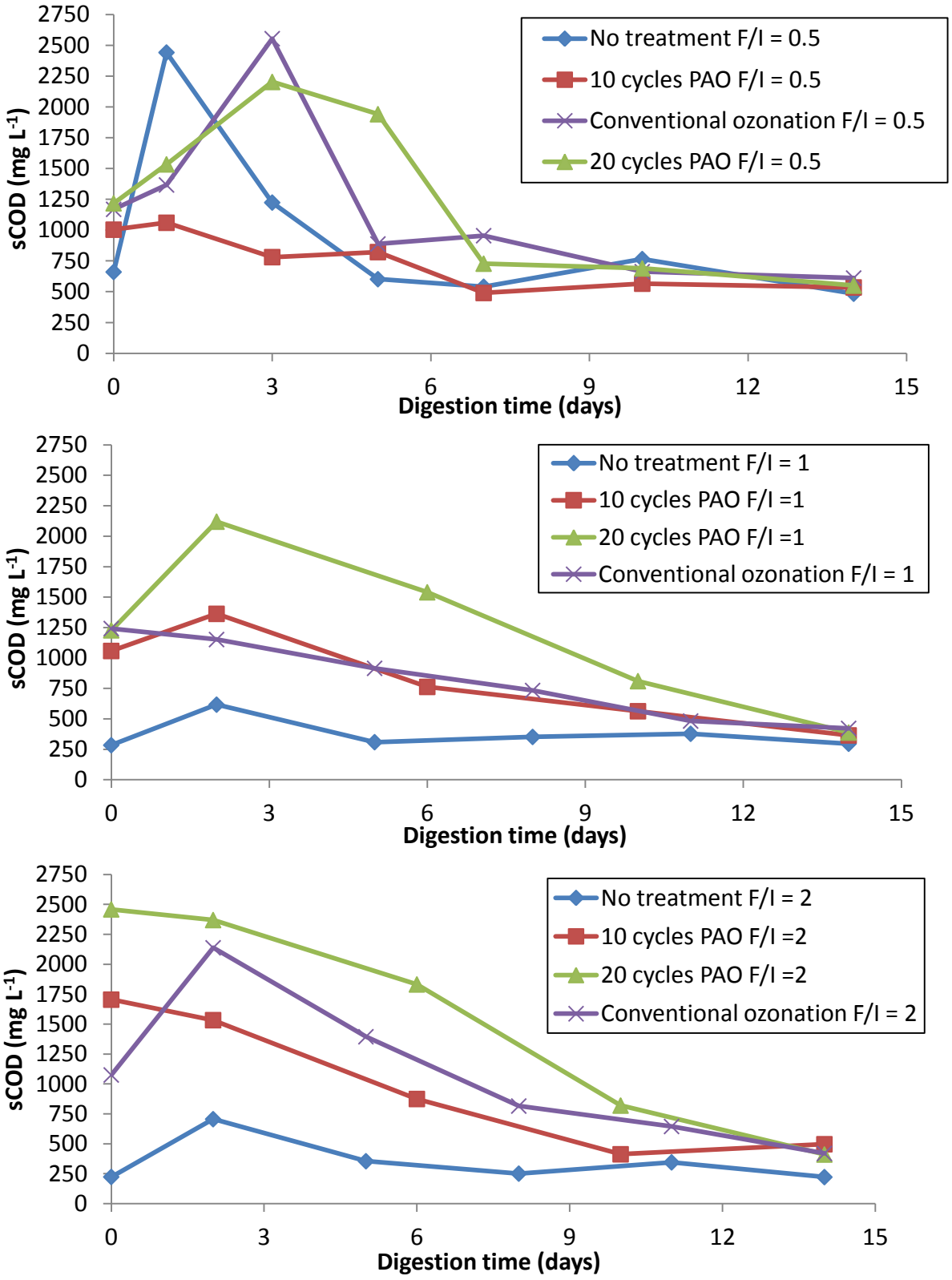


Figure 5.6 sCOD vs. time profiles at F/I = 0.5, 1, and 2

beginning of digestion without any initial sCOD increases in the first 5 days. Braguglia et al. (2006) reported similar results and suggested that the inoculum in the batch reactors was able to start utilizing sCOD for fermentation phase skipping the hydrolysis phase when initial sCOD was abundant. In this study, retarded solids reduction was observed when initial sCOD was high and its reduction was rapid, which appeared to be consistent with high initial sCOD made possible by cell solubilization via ozonation, which in turn cut short the hydrolysis process, resulting in less solids reduction.

5.2.1.3 Kinetics of anaerobic digestion

Figure 5.7 plots the initial specific substrate utilization rate (first 7 days) vs. initial available substrate, $-(d(COD_{AS})/dt)(1/COD_{inoc})$ vs. COD_{AS}). The available substrate is based on measured COD of the AS being fed as substrate in the anaerobic process, and the specific utilization rate calculated by division of the substrate utilization rate by the COD due to the inoculum being incubated that is assumed unchanged in the first 7 days. The specific utilization rate as normalized by the inoculum amount is warranted because the inocula have been varied significantly among experiments with various F/I ratios. The linearity in initial specific rates vs. initial available substrates indicates a first-order dependence on available substrate and this indicates, at the same time, a first-order dependence of the process (i.e., substrate COD removal) on the inoculum (i.e., the anaerobic bacteria population) because of the normalized utilization rate being used.

Within the narrow studied range of available substrate, the anaerobic digestion process is first-order with respect to the AS substrate feed and first-order

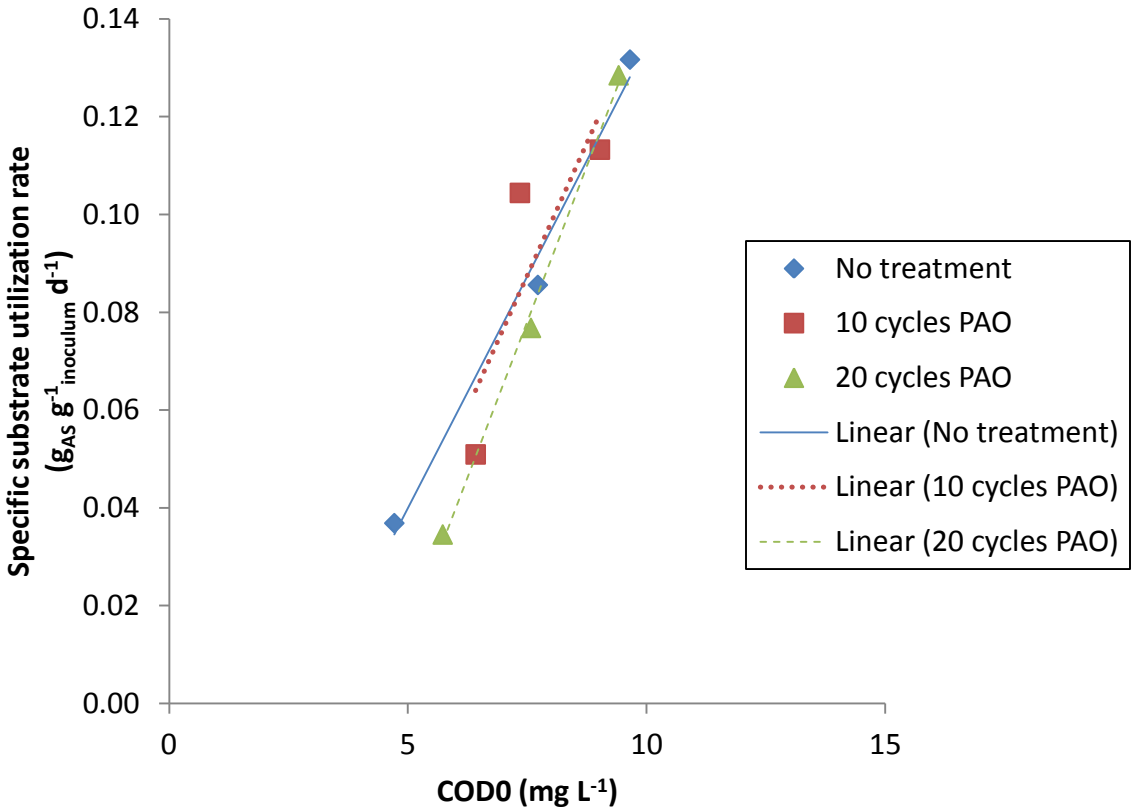


Figure 5.7 Linear plot of specific substrate utilization rate vs. available substrate

with respect to the inoculum present, thus being an overall second-order process for COD removal. By linear regression analysis, the slopes of these plots are found to be 0.019, 0.022, and 0.026 for sludge subjected to no treatment, 10 cycles of PAO, and 20 cycles of PAO, respectively. The second-order rate constants for digestion of the different sludge batches were quite similar within about 15% of each other, albeit with an increasing order with increasing ozonation treatment. The trend may have reflected a slight increase in the ability of the anaerobic bacteria in removal of COD offered by the ozonated sludge, but the effect of ozonation on digestion appears to be very modest.

A series of experiments were performed to examine the removal of COD according to varying the F/I ratios and AS feeds subjected to different treatments. The results are plotted as specific COD utilization rate vs. available COD, as shown in Figure 5.8. The rates have been calculated by taking an instaneous slope (the tangential line) from the monitored COD vs. time profile. The linearity of the specific utilization rate vs. available substrate again confirms for the anaerobic COD removal process as first-order with respect to the available sludge substrate and first-order with respect to inoculum amount. The slopes are second-order rate constants and found by linear regression analysis, as listed in Table 5.7. While the kinetics of digestion are similar for different treated substrate feeds indicating only a small dependence on treatment, they are significantly different at different F/I ratios. The dependence of kinetics (as specific rate) on F/I ratio is more complex and may be due to several interacting factors at work: the difference in available substrate to the consumers, the difference in species working on different substrate feeds, and the difference in the ranges of available substrates and consumers as differentiated by the experimental design (i.e., varying F/I ratios in batches while keeping the constant incubation volume)) (Braguglia et al. 2006; Gungor-Demirci and Demirer 2004; Ma et al. 2011; Moreno-Andrade and Buitron 2003; Neves et al. 2004).

Figure 5.9 compares the specific substrate utilization rates at different F/I ratios based on removals of COD, TS, TSS, and VS. In general, the specific utilization rates are consistent among different measures, e.g., higher COD removal rates corresponding to higher solid removal rates. A most salient point is the increase of specific utilization rate with an increase of F/I ratio from 0.5 to 1 and

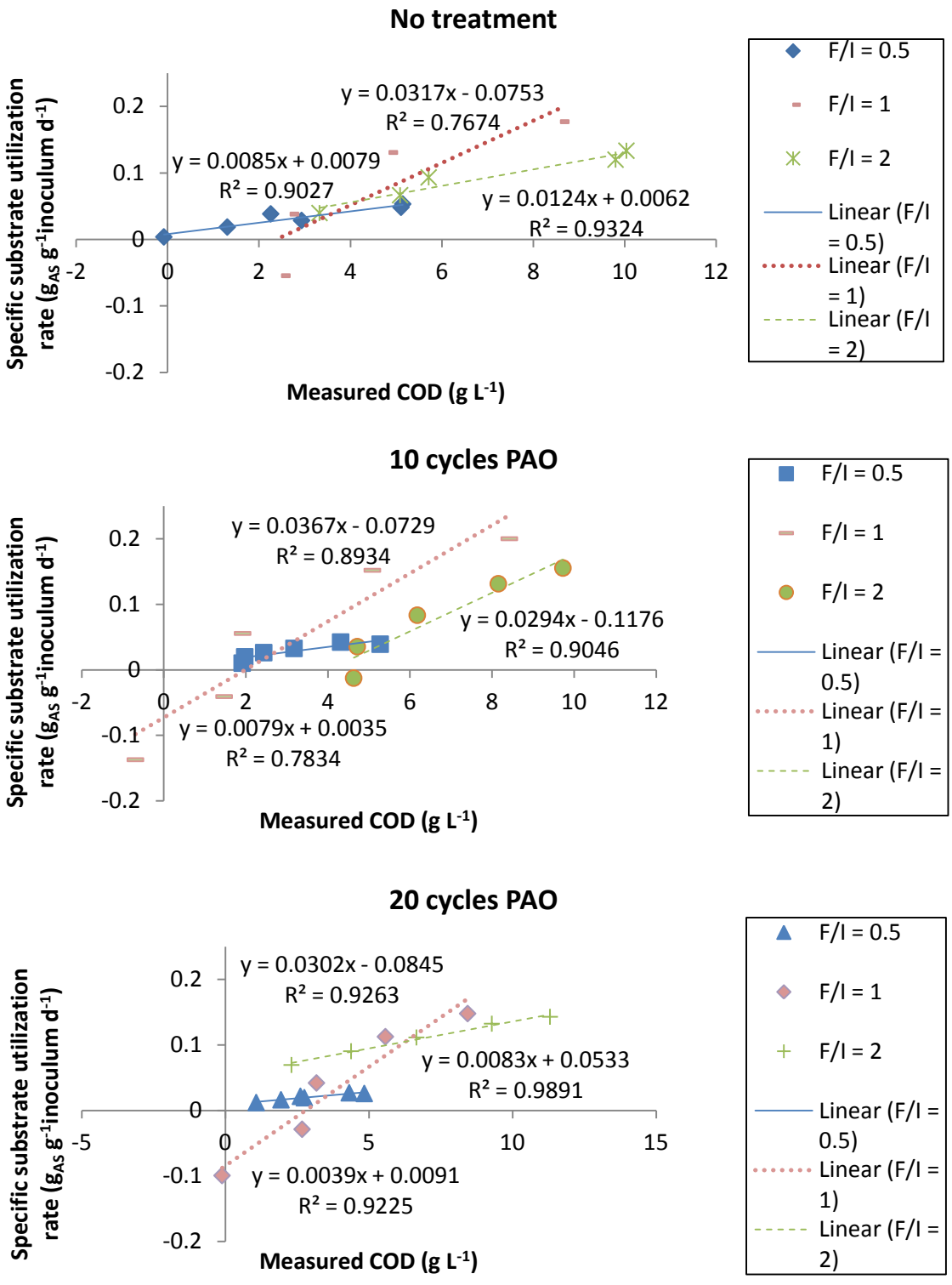


Figure 5.8 Kinetic equation fitting of COD_{AS} changes in anaerobic digestion tests with various pretreatments at F/I = 0.5, 1 and 2.

Table 5.7 Kinetic rate constant (L g^{-1} $COD_{inoculum}$ d^{-1}) for anaerobic digestion of AS at different F/I ratios

	F/I = 0.5		F/I = 1		F/I = 2	
	k'	R^2	k'	R^2	k'	R^2
No treatment	0.009	0.90	0.032	0.77	0.012	0.93
10 cycles PAO	0.008	0.78	0.037	0.89	0.030	0.90
20 cycles PAO	0.004	0.92	0.030	0.93	0.009	0.99

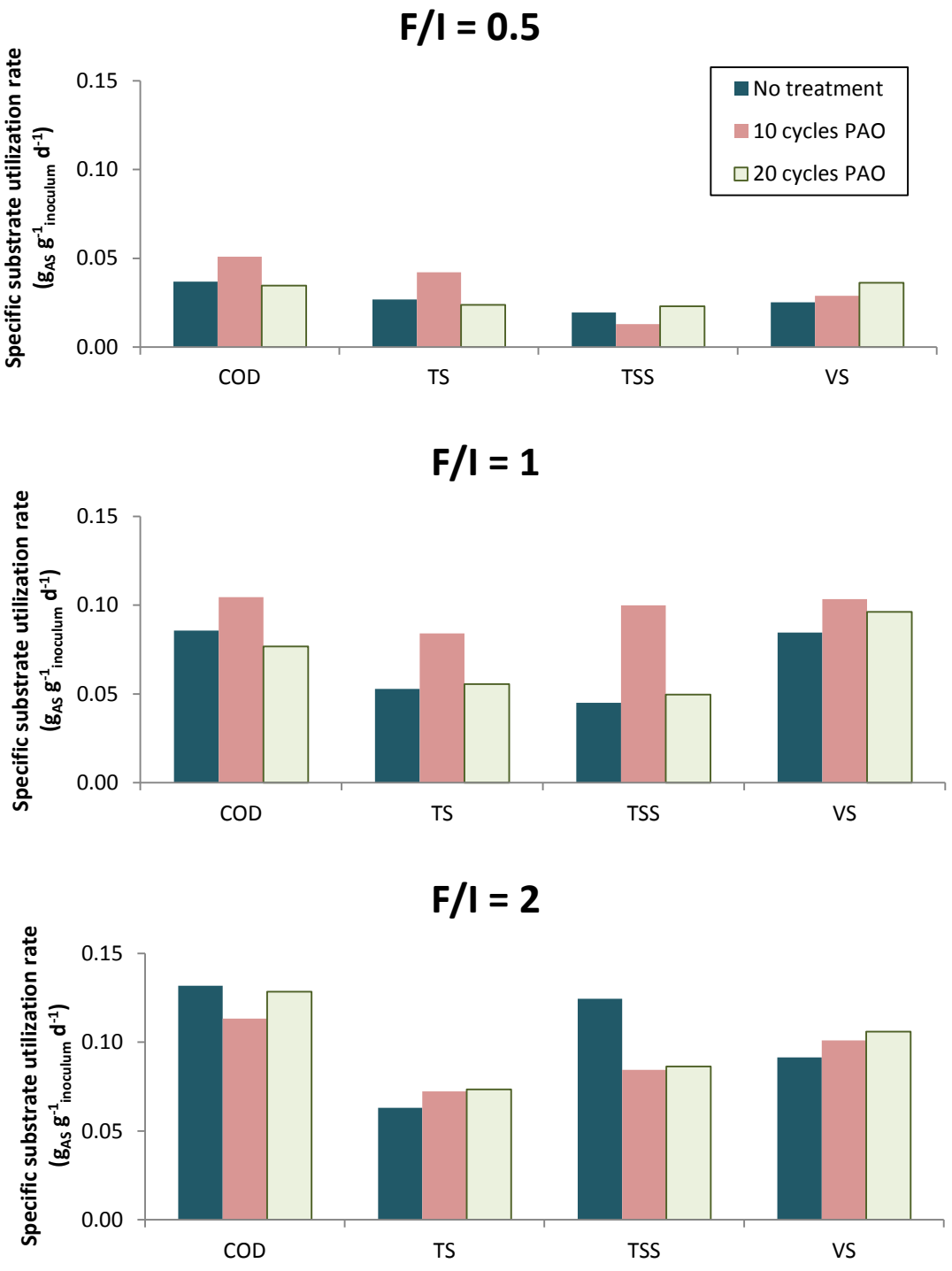


Figure 5.9 Specific substrate utilization rate (g substrate g^{-1} inoculum d^{-1}) as based on removals of COD, TS, TSS, and VS

then only a modest increase of specific rate for increasing F/I ratio from 1 to 2. This appears to reveal the Michaelis-Menten kinetics, where the kinetics increases with increasing substrate concentration but reaches a limit when the substrate becomes very abundant. However, it should be kept in mind possible interacting factors such as variations in the kinds of species and feeds as mentioned earlier.

5.2.1.4 Biogas production

The production of biogas was measured during anaerobic digestion at different F/I ratios and AS feeds treated differently. After 14 days of batch digestion, the cumulative biogas produced for untreated AS was 40, 82, and 111 mL incubated at F/I = 0.5, 1, and 2, respectively. When compared in specific gas production rates (mL biogas per g inoculum), the production rate increased significantly with increasing F/I ratios while the production rate at a specific F/I ratio was much less influenced by ozonation (Fig. 5.10). At lower F/I ratio of 0.5, ozonation of the feed AS exerted a positive effect on gas production, while no obvious effects were observed at the high F/I ratio of 2. Specifically at F/I of 0.5, increases of specific gas production rate by 160, 190, and 150% were determined for conventional ozonation (52 mg O_3/g TSS), 10 PAO cycles (5 mg O_3/g TSS), and 20 PAO cycles (10 mg O_3/g TSS).

5.2.1.5 Effects of ozonation on solids reduction and biogas production

The efficiency of anaerobic digestion as measured in solids reduction and biogas production appears to be strongly influenced by the F/I ratio rather than by ozonation. For gas production, ozonation has a modest positive effect at low F/I (0.5)

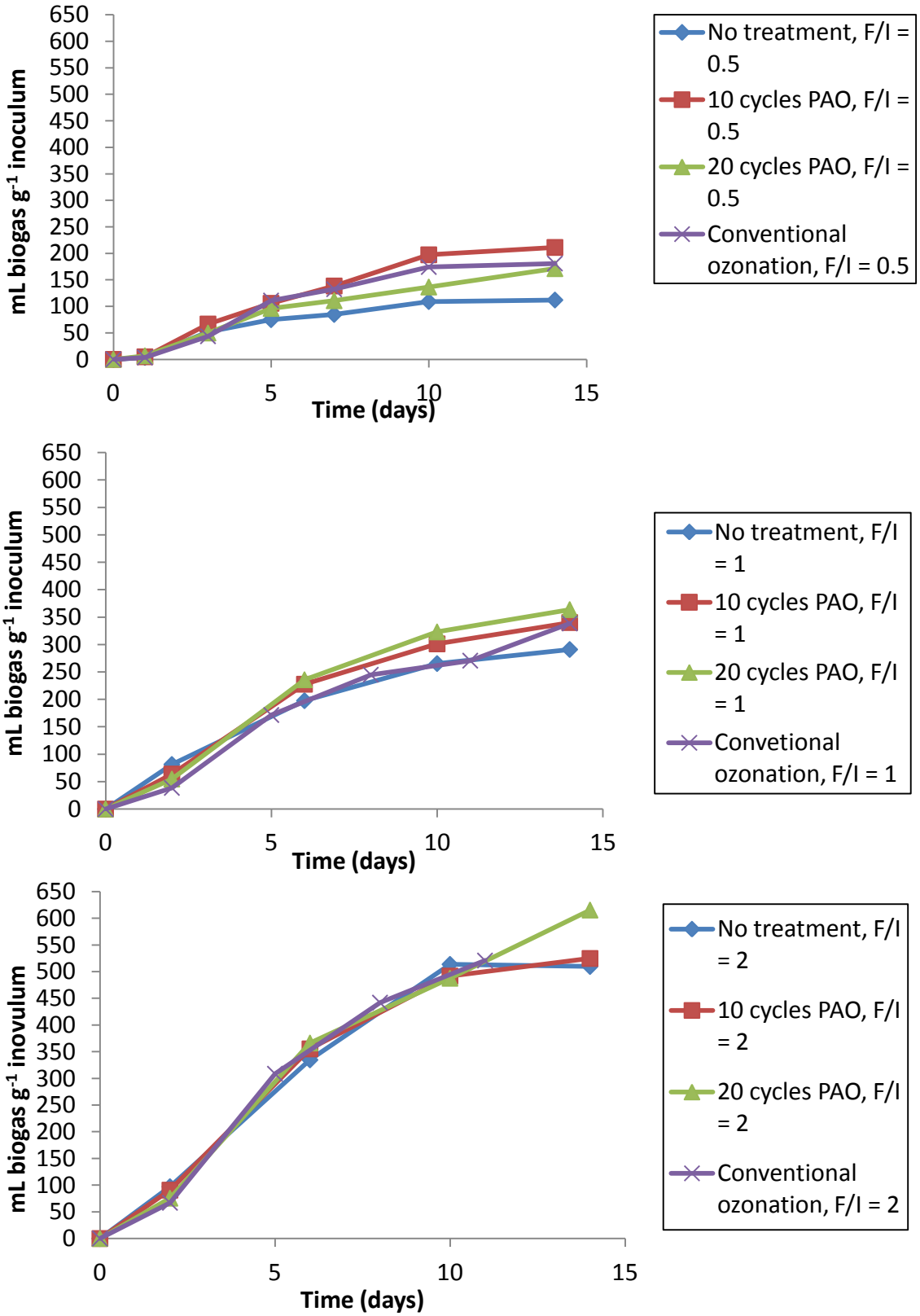


Figure 5.10 Specific biogas production of AS at different F/I ratios

but little effect at high F/I ratio (2). For solids reduction, ozonation shows a stronger positive effect over the entire 14-days period at high F/I ratios (1 and 2) (see Table 5.6) but little effect at low F/I ratio (0.5).

5.2.1 Anaerobic digestion with laboratory acclimated inoculum

5.2.2.1 Solids and COD reduction

Anaerobic digestion was carried out for 20 days with acclimated inoculum (acclimation as described in Chapter 4 on Methods). Figure 5.11 shows the suspended solids and COD reduction (%) after 20 days of anaerobic digestion of RAS subjected to PAO and conventional ozonation and under different F/I ratios. The results were complex. When F/I was low at 0.4, higher TSS and VSS reduction resulted with RAS treated with 20 cycles of PAO than RAS without treatment, while COD reduction was similar in both. When F/I was high at 0.8, TSS and COD reductions of PAO-treated RAS were similar to untreated RAS, while VSS reduction of PAO-treated RAS was higher than untreated RAS. Additionally, at F/I of 0.4, solids and COD reductions of RAS subjected to conventional ozonation were even lower than untreated RAS, while SS reduction increased to similar levels of untreated RAS.

Ozonation was reported to assist digestion of particulate RAS via flocs disintegration (Cheng et al. 2012; Chu et al. 2009a; Zhang et al. 2009). Our results of higher TSS and VSS reduction in PAO-treated RAS with lower O_3 dose (10 mg O_3 g^{-1} TSS) would support the physical disintegration of the flocs resulting in more "disrupted" solids that are more amenable to digestion than untreated "whole" RAS.

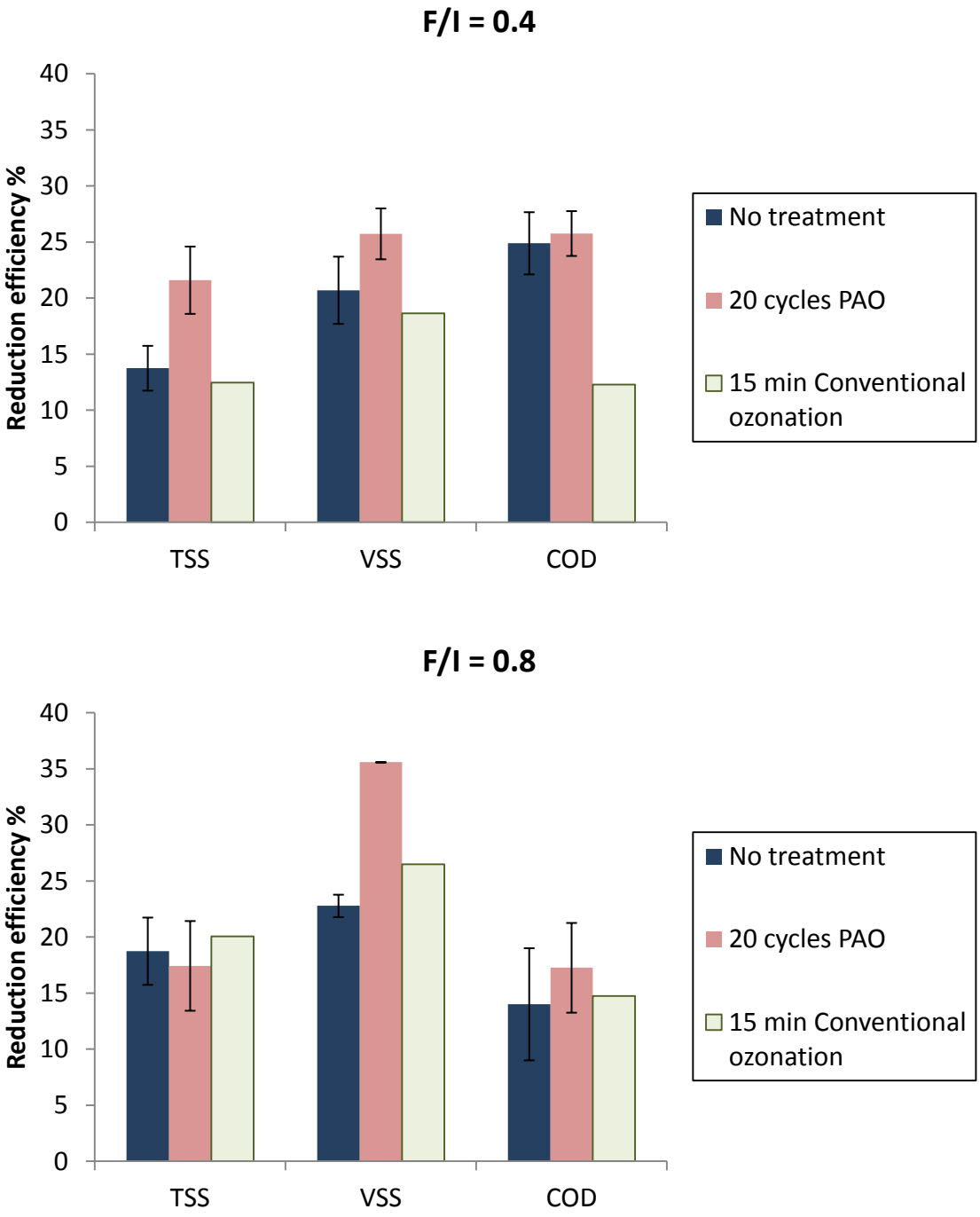


Figure 5.11 Suspended solids and COD reduction efficiencies (%) after 20 days of anaerobic digestion under F/I ratio of 0.4 and 0.8 g (TSS_0 = 4.8-6.1 g L^{-1}, VSS_0 = 3.9-5.1 mg L^{-1}, COD_0 = 7.2-7.9 g L^{-1}, Inoculum = 3-5 g L^{-1}. See Table 4.2)

However, when the ozone dose was increased from 10 to 50 mg O_3 g^{-1} TSS by means of conventional ozonation, refractory materials were reported to influence solids reduction (Bougrier et al. 2006). Also reported was that negligible VS removal resulted after 10 days of anaerobic digestion when the feed had been treated by conventional ozonation at 50 g O_3 g^{-1} TS (Braguglia et al. 2012). When the F/I was increased from 0.4 to 0.8, increasing VSS reduction was observed with ozonated RAS with and without pressure cycles, suggesting more biodegradable solids were made available and digested. The results of Section 5.2.1 showed that, despite a smaller dose delivered by 20 cycles of PAO that was 1/5 of conventional ozonation (Table 4.2), higher solids and COD reductions were possible. Moreover, while COD reductions of PAO-treated RAS and untreated RAS were similar when F/I was 0.4, higher VSS reduction of PAO-treated AS resulting in higher COD reduction would be expected if longer SRT were used.

5.2.2.2 Soluble COD reduction

Figure 5.12 shows sCOD reductions after 20 days of anaerobic digestion of RAS subjected to PAO and conventional ozonation. For untreated RAS at F/I = 0.4, little sCOD removal resulted after 20 days, indicating little utilization by the subsequent methanogenic phase (Braguglia et al. 2006). The trends of sCOD removal were similar for PAO and conventional ozonation treatments. More sCOD was removed when F/I was increased from 0.4 to 0.8. Although the initial sCOD of PAO-treated RAS was higher than conventionally ozonated-RAS (Table 4.2), higher sCOD removal resulted in the PAO-treated feed. As cited above, refractory compounds were reported when conventional ozonation was applied prior to

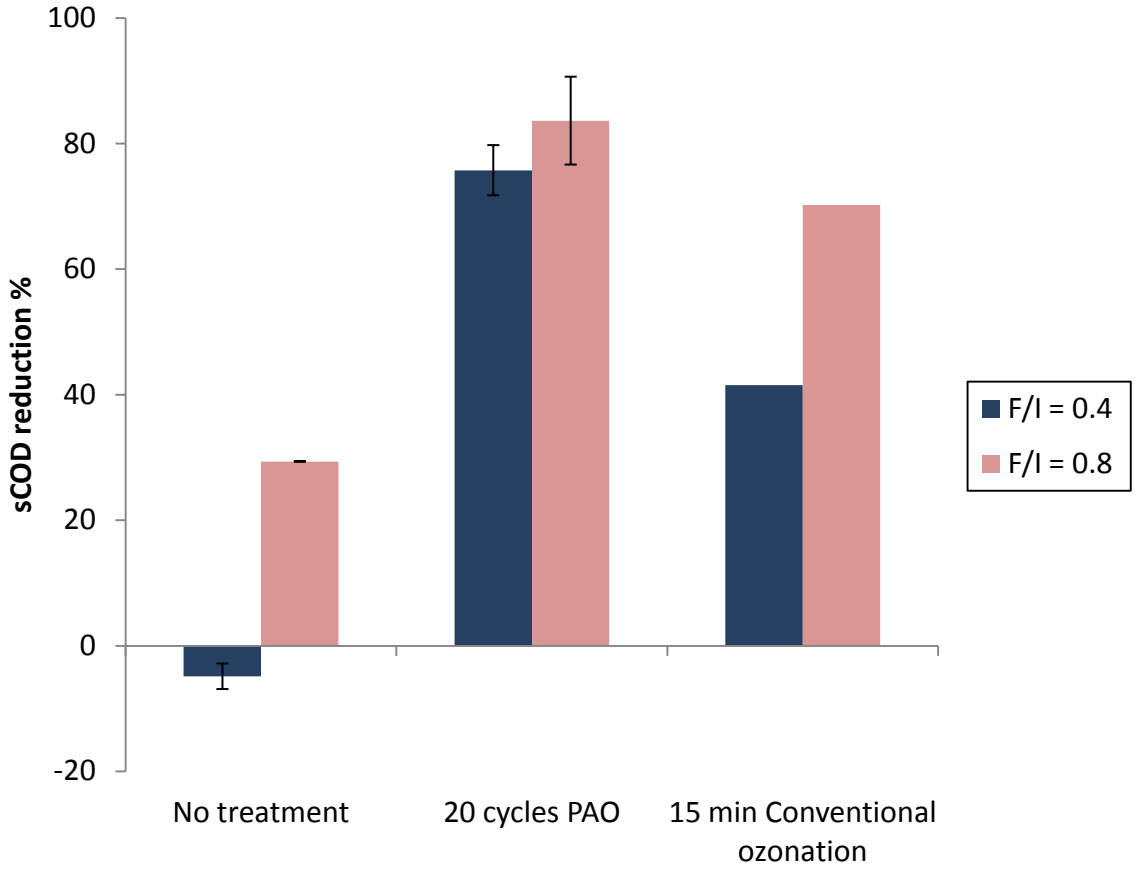


Figure 5.12 sCOD reduction (%) after 20-days digestion of untreated and treated RAS at F/I ratios of 0.4 and 0.8

anaerobic digestion, which was attributed to refractory compounds interfering with not only solids and COD removal but also with anaerobic microbial activities (Bougrier et al. 2006). Unlike conventional ozonation, higher solids and sCOD reduction using PAO at F/I of 0.4-0.8 may be indicative of an advantage of PAO in circumventing the hydrolytic phase of digestion thus accelerating the process to the methanogenic phase (Braguglia et al. 2006).

5.2.2.3 Biogas production

Fig. 5.13 shows the specific biogas production (mL biogas g^{-1} COD supplied) during anaerobic digestion of untreated and ozonated RAS. When F/I was low at 0.4, specific gas productions were similar among untreated and ozonated RAS. The differences in specific gas production were amplified when F/I was high at 0.8. At F/I of 0.8, the specific biogas production of RAS subjected to 20 cycles of PAO and conventional ozonation were 9- and 3-fold, respectively, higher than the untreated RAS.

While the differences in specific biogas production of treated and untreated RAS were small at F/I of 0.4, TSS and VSS reductions of the PAO-treated RAS were more pronounced than the untreated RAS (Figs. 5.11 and 5.13). It appears that at low F/I, PAO assists solids reduction in a more meaningful fashion by which the anaerobes are able to access solubilized materials, thus shortening the hydrolytic step. At F/I ratio of 0.8, higher VSS reduction and specific biogas production (Figs. 5.11 and 5.13) were observed with PAO-treated RAS, suggesting that PAO enhanced solids reduction and biogas yield at the same time at high F/I of 0.8 in which abundant, accessible substrates are available. In contrast, the higher O_3 dose via conventional ozonation delivered lower COD solubilization (Table 4.2) and might have resulted in refractory products that hampered VSS reduction and biogas production.

5.2.2.4 Effects of ozonation on acclimated inoculum in solids reduction and biogas production

Ozonation treatment of RAS impacted acclimated inocula more positively for

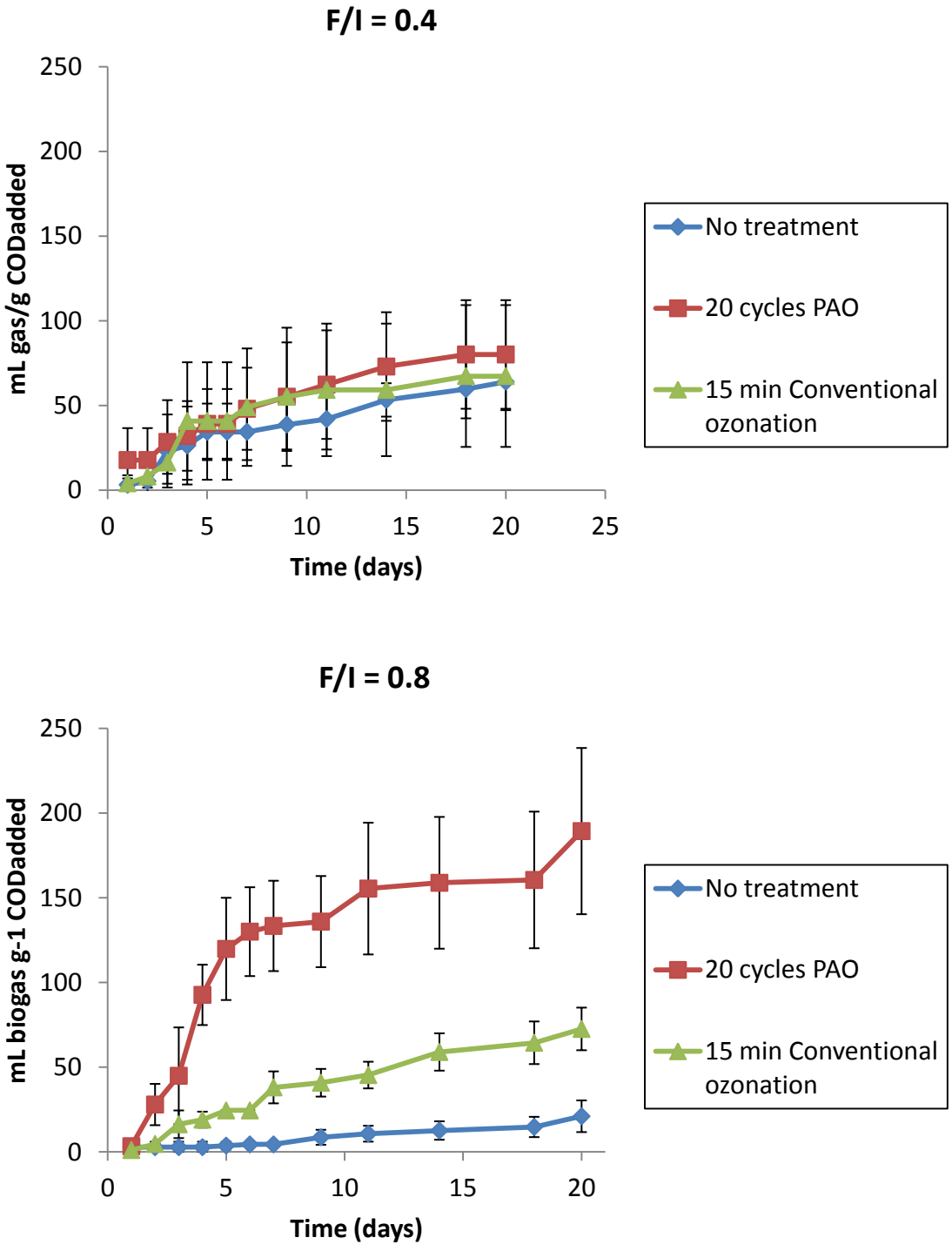


Figure 5.13 Biogas production during anaerobic digestion with acclimated inoculum at F/I ratios of 0.4 and 0.8 of untreated and treated RAS

solids reduction and biogas production. The 20 days digestion results showed that solids reduction and biogas production were more significantly enhanced for acclimated inocula than those obtained just prior to use. The solids reduction and biogas production results of RAS treated by conventional ozonation were improved by 16% in VSS reduction and by 240% in biogas production, comparable to improvements reported in the literature (Carrere et al. 2010; Goel et al. 2003). At F/I range of 0.4 and 0.8, PAO treatment demonstrated higher efficiency in solids reduction and biogas production with a smaller O_3 dose. Related studies have shown that pressurization-depressurization cycles were useful for disrupting AS and other organic compounds physically and chemically (Cheng et al. 2012; Hong et al. 2008b; Ma et al. 2011). This study further showed the potential of repeated pressurization-depressurization cycles with O_3 to disrupt and increase accessibility of cell materials for the subsequent anaerobic digestion process.

CHAPTER 6

CONCLUSIONS

Based on the experimental tasks and results obtained, the following conclusions are made. Ozonation of AS by rapid, successive compression-decompression cycles resulted in sludge disintegration, solids reduction, and soluble organic matter increase (soluble TN, DNA, protein, and TP). While TSS was significantly reduced when RAS was treated by ozone with and without pressure cycles, a good mass balance of solids was obtained that indicated insignificant TS mineralization by ozone ($p = 0.05$). Conventional ozonation (bubbling ozonation) at 77 mg O_3 g^{-1} TSS over 15 min resulted in TSS reduction by 12% and sCOD increase by 9.1%. In comparison, ozonation in 20 pressure cycles ($P = 1040$ kPa) at a smaller ozone dose of 10 mg O_3 g^{-1} TSS over 16 min resulted in greater TSS reduction by 25% and sCOD increase by 21%. Ozonation with expanding microbubbles made possible by rapid pressure cycles was advantageous over a conventional bubble contactor in terms of ozone utilization and sludge solubilization.

Digestion results using inocula from WWTPs just prior to use showed solids reduction to be significantly dependant on F/I ratios in the range 0.5 to 2. Ozonation exerts a stronger positive effect over the entire 14-days digestion period at high F/I ratios (1 and 2) but little effect at low F/I ratio (0.5). For gas production, ozonation has a modest, positive effect at low F/I (0.5) but little effect at high F/I ratio (2).

Digestion results using acclimated inocula showed significant improvements in solids reduction and biogas production by conventional ozonation and PAO. Using conventional ozonation and F/I = 0.8, VSS reduction and biogas production were improved by 16% and 240%, respectively. Using PAO at a smaller dose (1/5 of conventional) at F/I of 0.4 to 0.8, VSS reduction and biogas production were improved by 24 and 57% (F/I = 0.4 and 0.8) and 25 and 800% (F/I = 0.4 and 0.8), respectively.

This study has demonstrated the advantage of using PAO in terms of improved solids reduction and biogas production during anaerobic digestion.

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